IN VIVO IMPLANTATION OF THE MANDIBULAR CONDYLE

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An experimental investigation of the growth of the lower jaw

PROMOTOR: PROF. DR. F.P.G.M. VAN DER LINDEN

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS DR. A.J.H. VENDRIK,
HOOGLERAAR IN DE FACULTEIT EN DER GENEESKUNDE
EN DER WISKUNDE EN NATUURWETENSCHAPPEN,
VOLGENS BESLUIT VAN DE SENAAT IN HET OPENBAAR TE VERDEDIGEN
OP VRIJDAG 26 MEI 1967 DES NAMIDDAGS TE 2 UUR

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HERMAN SEBASTIAAN DUTERLOO

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THOBEN OFFSET NIJMEGEN

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From the Department of Orthodontics (Head: Prof. Dr. F.P.G.M. van der Linden)
School of Dentistry, Faculty of Medicine, University of Nijmegen, Netherlands.

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is gratefully acknowledged.

To my parents
To Sofia, Eline and Servaas

PREFACE

Present knowledge of the growth and development of the craniofacial skeleton is far from complete. In particular, little is known about the factors regulating these processes. The problems lying here are not of merely theoretical importance, but are increasingly demanding the attention of the oral surgeon and of the orthodontist, both of whom in practicing their professions have regularly to do with handicapping deformities of the human face.

In this field too, fundamental knowledge is the essential basis for the advancement of therapeutic methods.

The experimental investigation presented here is intended as a contribution to the analysis of factors determining the growth of the mandible.

CONTENTS

Chapter 1

GENERAL INTRODUCTION

A. METHODS OF INVESTIGATING CRANIAL GROWTH

The cranio-facial skeleton is a complex entity and its growth can be studied in many ways. Besides examining human material, people have from olden times made use of animals to investigate this phenomenon. Especially when fundamental processes form the focus of interest, the use of experimental animals provides great advantages. Not only is it possible to apply techniques that cannot be permitted with human subjects, but one can also more satisfactorily combine different procedures in one experimental design. A well-planned poly-methodical approach may, through integration of its findings, yield a larger body of information and enables one to arrive at more far-reaching conclusions than a mono-methodical approach.
Before making a theoretical digression into the processes of cranial growth we shall deal briefly with those methods that have contributed most to our knowledge in this field.

The introduction of the standardized teleroentgenocephalometric technique by Pacini in 1922 opened a new era for the study of the human skull. Not only did this method enable workers to determine unequivocally the internal skeletal structures and to investigate their relationships exactly, but baved the way for the longitudinal study of living individuals during growth.(Broadbent 1937,Brodie 1941). Björk's (1955, 1963) application of the ancient technique of metal implants to human material made it possible to arrive at more precise conclusions as to sites, directions and amounts of growth.

An older, qualitatively oriented technique for the study of growing bone is vital staining. In the first half of the 18th century Belchier (1736) and Duhamel (1742) demonstrated the staining of newly-formed bone by the feeding of madder to animals. Duhamel studied the growth in the shafts of long bones and stated that 'a bone grows like tree and the periosteum

is the cambiumlayer'. It remained for John Hunter (1771), thirty years later, to point out that deposition was always accompanied by resorption so that bones 'were always kept in their just proportions as they grew'. All morphological changes take place on surfaces, there is no interstitial growth of bone.
One of the derivatives of madder is still used as a bone marking agent by injection in the synthetic form of Alizarin Red S. Of recent years other bone marking agents have come to the fore, of which tetracyclines and lead acetate are in regular use. When bone has been stained with a vital dye, its qualitative as well as its quantitative changes can be studied. The information thus obtained has, to a limited extent, a longitudinal character. This vital staining technique has been employed by many. By its means the cranial growth of various species has been investigated. Side by side with the vital staining technique the classic histological techniques have been used for years, of course, to study growth changes at microscopical level. The detailed morphological information thus acquired is considerably enlarged when this technique is combined with that of autoradiography. In the latter, substances labelled by such isotopes as S^{35}, Ca^{45}, C^{14}, P^{32}, or H^{3}, are administered, find their way into the metabolic pathway, and can afterwards be located in the microscopic sections. Both a qualitative and a quantitative approach are possible. Combined this has made the study of cell population kinetics possible; in a limited way these techniques have been applied to parts of the growing skull.
An entirely different approach to the study of the growth of the craniofacial skeleton is the one in which surgical intervention is used. Here the techniques indicated above can be applied as auxiliary resources.

Experimental embryology, e.g. the *in vitro* culture of skeletal parts, which has yielded much information in the morphogenetic and cellular-physiological fields as has also the *in vivo* implantation technique. In the latter a study is made of the development of whole or partial skeletal parts implanted at various ectopical sites in the same individual or in a conditioned host.
In the present investigation the *in vivo* implantation technique has been employed. For this reason a detailed discussion of it will follow later on.

B. THEORIES OF THE MECHANISM OF CRANIAL GROWTH

A larger number of investigators have devoted themselves to the study of the growing skeleton. But until a few decades ago, however, our insight into the complex processes regulating the growth of the skull remained very limited and did not penetrate very far beyond what Hunter (1771) had already pointed out. This was especially true as regards postnatal growth. Seen in this light, the work done in the 20's by Brash (1924) and Keith and Campion (1922) was of vital importance. They attempted to determine what they called 'mechanisms of growth'. Among other things they arrived at the important conclusion that only an analytical approach could solve the many problems. By this they meant that the study of the growth of separate components would lead to a better insight into the general process. Since then a considerable research has been done which has resulted in various interpretations.

The bones of the skull are classified as membranous and cartilaginous according to their origins. The bones forming the cranial vault and the facial skeleton are termed 'membranous' because they originate from an embryonic membrane through direct differentiation. At various sites in these membranes, centres of ossification occur, and spread. Where the ossified fields meet, sutures are formed. The surfaces of these sutures are covered by a layer of osteoblasts. Weinmann and Sicher (1955) stressed the importance of the proliferating intersutural connective tissue between these layers. They held this tissue responsible for the spreading of the membranous bones, to which they attach essential importance for the growth of the skull, since they see in it the remnant of the original embryonic membrane. It is the proliferation of the intersutural connective tissues which, they allege, initiates sutural bone growth. They postulate that this proliferation is, ***inter alia***, responsible for the enlargement of the neurocranium, thus enabling the brain to grow.

Weinmann and Sicher (1964) consider the condylar cartilage very important for the growth of the mandible. As they put it: 'Normal proliferation of the growth of the mandibular condyle is not only directly responsible for the overall enlargement of the mandible but also indirectly for the normal vertical development of the entire face and for the normal vertical eruption of the teeth'.* To express similar opinions

* Weinmann, J.P., Sicher, H. (1964): Histophysiology of the temporomandibular joint. The temporomandibular joint. (ed. B.G.Sarnat), p.76.

other workers have used such terms as 'pacemaker' and 'organizer' (Sarnat and Laskin, 1954, Baume 1962) or 'chief site of growth' (Brodie 1964).

Another theory has been proposed by J.H. Scott (1953, 1956, 1958, 1962). According to him the sutural connective tissue contains blood vessels and connecting fibres and the sutural edges are covered with a proliferating bone-forming layer. These layers grow independently of each other. The spreading of the bones connected by a suture is, in Scott's view, due to extra-sutural influences, for instance the growing brain, the eyeballs, and the growing cartilage of the basal synchondroses and nasal septum. The cartilaginous structures, in particular, are considered important by this investigator. Thus he sees the cartilaginous nasal septum as a chief factor in the growth of the maxilla, a view shared by Sarnat and Wexler (1966). As regards the significance of the condylar cartilage for the growth of the mandible, here Scott agrees with Weinmann and Sicher, and the other workers mentioned.

Wether over the entire period of growth the same value should be attached to the functional significance of the structures under discussion, is not quite clear. Weinmann and Sicher think that sutural growth plays an important part over the whole period of the postnatal growth process. Scott, on the other hand, assumes that for man this is only true for the first six years of his life, and that during this period the brain, the eyeballs and the cartilaginous structures have a dominating influence.

It is generally held that over the entire period of growth the cranial bones undergo local modifications as a result of differential deposition and resorption processes. Recently, Enlow *et al.* (1962, 1963, 1964, 1965, 1966) have made a detailed study - both in man and in animals - of the patterns according to which this happens in the facial skeleton.

The bones of the cranial base have been termed cartilaginous because at the embryonic stage, ventrally from the brain, a cartilage model is laid down which is called 'chondrocranium'. Remnants of this, even after the ossification centres have made their appearance, continue to exist. They constitute the basal synchondroses, and it is here that the development of the cartilage is continued. Next, this cartilage, by the process of endochondral ossification, is transformed into bone, (In man the synchondroses are ultimately replaced by bone). Weinmann and Sicher (1955) attach to the growth occurring in these synchondroses a similar importance as to that occurring in the sutures.

As is the case for neurocranial growth, Weinmann and Sicher hold the sutural growth also responsible for the development of the facial skel-

eton. In their view it is the sutural growth which causes the development of the facial skull forwards and downwards in relation to the cranial base. Such a movement would be possible because of the direction in which the sutural surfaces are oriented.
The mandible develops within a membrane lateral to and at some distance from Meckel's cartilage. As with other membranous bones, mandibular development is complicated by the formation of so-called secondary cartilages. Symons (1952, 1965) defines them as cartilages having no topographic connection with the chondrocranium. They have their origins at a much later stage and are only of short duration. Their number, relative size, and life span are different for the various species.

Condylar cartilage is one of the secondary cartilages. Compared with the others, it is of major importance and has a longer life span. According to Weinmann and Sicher, condylar cartilage develops at the end of the condylar process as the result of a differentiation in the deeper strata of the covering connective tissue membranes. This, they think, explains why the articular surface of the mandibular joint consists of a layer of connective tissue. They also are of opinion that, unlike the epiphyseal and articular cartilage in other joints, condylar cartilage does not grow interstitially but solely through deposition. From the deeper strata of the upper connective tissue layer new cartilage cells would differentiate.

The theory of the functional cranial components has an entirely different starting-point. Originally, this theory was developed by van der Klaauw, (1946), after a detailed comparative zoological study. Moss *et al.* (1959, 1960, 1962, 1964) have recently worked out his idea. They view the skull from a different angle. About the cranial bones Moss states: 'The form of these bones (where FORM = SIZE + SHAPE) as well as their position in space, have no necessary (obligatory) relationship to each other. They are, in fact, relatively independent, both phylogenetically and ontogenetically'.* In the head, cartilage as well as bone would fulfil their secondary task of supporting and protecting tissues. According to this concept the form of the cranial bones depends on their functional matrix. This latter term, introduced by Moss, is defined by him as 'a convenient term to give to all the soft tissues related to a given skeletal element'.**

* Moss, M.L. (1962): The functional matrix. Vistas in orthodontics (ed. B.S.Kraus, R.A.Riedel), p.85.
** Idem p.88.

He sees the functional cranial components as 'embedded within their functional matrices'. It should be realized here that, for example, it is not the brain which is referred to, but the contents of the neurocranium. Not the eyeball, but the contents of the orbit constitute the functional matrix. In the terminology of Moss the functional matrices of the head are formed by the neural mass, orbital mass, muscles, glands, dentition, neurovascular bundles, and the nasal-, oral-, and pharyngeal cavities. It is remarkable that Moss should attribute to the cavities, as empty spaces, hence as physiological, functional but non-structural realities, a morphogenetic significance: 'The empty space of these cavities (nasal, oral, pharyngeal) are biologic realities, indeed their hollow patency is prerequisite to their normal function'.*

As regards the growth of skeletal elements, Moss assumes that this, too, is mainly dependent on the growth of their functional matrix. This means that extrinsic factors, that is, factors outside the skeletal tissue, regulate the growth and maintenance of the forms of the skeletal tissue in the head. The development of the skeleton at the embryonic and foetal stages, on the other hand, is strongly subject to intrinsic factors, which in this case are present in the skeletal tissue itself. According to Moss, these factors are only of primary significance over a short period.

Despite the fact that the views of Scott and Moss differ in many respects, neither of them - unlike Weinmann en Sicher - attributes to sutural growth anything more than a secondary, adaptive significance. Nor has Moss any wider claims for the cartilaginous structures, to which Scott attaches a good deal of importance. Moss thinks that it is the growth of the soft tissues which makes the cranial bones spread.

As for the significance of the cartilage of the mandibular condyle for the growth of the mandible, Moss holds a different view than the other workers mentioned. He postulates that at the period of growth the mandible is situated in a developing functional matrix, whose growth is also responsible for the growth of the mandible. According to him this implies that the processes occurring in the mandibular condyle are of an adaptive character: 'Growth of the condylar cartilages is not the primary force of this bone. Growth here as at the neurocranial suture areas, is a secondary response to the growth of viscera'.**

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* Moss, M.L. (1964): Vertical growth of the human face. Amer.J.Orthodont.50, p.371.

** Moss, M.L. (1962): The functional matrix. Vistas in orthodontics (ed.B.S.Kraus, R.A.Riedel), p.93 and 94.

C. MAIN PROBLEMS - PLAN OF INVESTIGATION

The aims of the present investigation has been in the first place to obtain a better insight into the growth of the mandibular condyle. As has been said above, views are widely different about the significance of the processes of growth in the mandibular condyle. Weinmann and Sicher and Scott, hold the growth in the condyle directly responsible for the growth of the mandible and indirectly for the growth of an important part of the viscerocranium. According to them, the processes of growth in the mandibular condyle occupy a key position here. Moss represents the contrary view that condylar growth is very much dependent on the growth of the soft tissues.

The essential difference between these views lies in the emphasis on the significance of the influence of the intrinsic and extrinsic factors by the various authors. In this case the intrinsic factors are those present in the tissues of the mandibular condyle itself. In this connection the view defended by Weinmann and Sicher (and also by Scott) may be interpreted in the sense that it is the intrinsic factors which are the essential ones for the growth of the mandibular condyle. Condylar growth is alleged to be largely determined by it.

Moss, on the other hand, submits that such intrinsic factors are only of importance in the initial phases of the ontogenesis, but that afterwards mandibular growth is dominated by the extrinsic factors, situated outside the skeletal tissues.

Investigations into the interaction of such factors in skeletal tissues have been carried out in particular by means of *in vitro* cultures of embryonic long bone anlagen (Fell, 1956) and by *in vivo* implantation of foetal and postnatal long bones (Felts, 1959, 1961). These and similar experiments have shown that even in such an isolated situation elements of the skeleton are capable of remarkable development. This has been interpreted as the result of intrinsic factors. It appeared, however, that various minor deviations occurred and that some stages of development failed to take place. From this it was inferred that only a normal interaction of intrinsic factors and extrinsic factors would create the circumstances leading to a normal form and development.

In the growth of such young parts of the skeleton, chondrogenesis plays an important part. This is also true for mandibular condyle growth. The secondary cartilage which is developing here, has an entirely different philogenetical background, however. The ontogenesis and the histological built-up of the mandibular condyle, too, are different from those of the long bones and their articular cartilages.

Hence the aims of our investigations have been directed towards the question of whether chondrogenesis in the mandibular condyle is dominated by the intrinsic and the extrinsic factors in the same or in a different way as in the case in a long bone. For this purpose we performed a number *in vivo* implantation experiments.*

Only a few *in vivo* implantation experiments with mammalian mandibles or parts of them have been reported in the literature, and none of them referred specifically to the problem just mentioned.

More specifically, our problem may be formulated as follows:

1. Is the isolated mandibular condyle (i.e. when taken outside its normal environment, as in the case of *in vivo* implantation) still capable of exhibiting perceptible growth?
2. If such growth should occur, is it comparable to that found in the normal situation?
3. If chondrogenesis should take place in the implanted mandibular condyle, does it differ - and if so, how - from chondrogenesis occurring in an implanted long bone?

In the next chapter the materials and methods used are described, and in Chapter 3 a number of *in vivo* implantation experiments with epiphyseal costal cartilage. The purpose of these experiments was to put the techniques and the site of implantation to the test in order to arrive at the best possible experimental procedure. Chapter 4 deals with the *in vivo* implantation of the third metacarpal bone; in Chapter 5 a similar experiment with the mandibular condyle is described. For a better interpretation of our findings it seemed desirable to carry out a number of supplementary *in vivo* implantation experiments of the mandibular condyle in combination with the injection of tetracycline and tritiated thymidine. These experiments are described in Chapters 6 and 7. In addition, Chapter 8 reports on experiments in which the implantation period of the mandibular condyle was extended. Chapter 9 discusses briefly the behaviour of implants introduced elsewhere. After a general discussion (Chapter 10) the book ends with a Summary.

* The terminology for this type of experiments, as used in this thesis, was suggested by Transplantation Bulletin 1955, Waverly Press Baltimore.

Chapter 2

MATERIALS AND METHODS

A. THE ANIMALS

1. *General information.* For the experiments described in the present thesis no other than Wistar-strain rats were used. The animals were kept in the Central Animal Laboratory (Head: Dr. vet. J.M. Dobbelaar) of the Faculty of Medicine, University of Nijmegen, Netherlands (Dobbelaar, 1960). As we needed a large number of young animals, their breeding was done in the Laboratory. The parents had been provided by 'Centraal Proefdierenfokbedrijf TNO', Zeist (Netherlands).

   For the *in vivo* implantation experiments 4-day-old rats were used. At that age the animals are about 6 cm long (not including the tail); the head is about 2 cm. They still have a foetal appearance and are hairless. Eyes and ears are closed by periderm. Nevertheless, they already lend themselves very well to experimental intervention: the animals are easily handled, possess great resistance, and generally are not much liable to infection. Pilot studies had indicated that a 7-day experimental period would satisfy requirements. In the interval between the 4^{th} to 11^{th} days the rats grow very quickly. Their body weight is nearly doubled. Skeletal development, too, over this period is very marked. Skull and jaws undergo major changes as regards shape and dimensions. At four days the mandibular joint has reached full development and has become operable. Form and size of the mandibular condyle make an *in vivo* implantation feasible. Another reason why the experiments were carried out with 4-day-old rats was the the fact that at this age the junction of the condylar cartilage with the bone of the ascending ramus is fairly smooth, and not arched, as at a later stage.

   A drawback of such young animals in long-term experiments is that when the intervention has been performed, the young animal has to be put back with its mother. Rejection of the offspring and cannibalism may be the result. Initial difficulties in this respect were, however, overcome to a considerable extent.

2. *The breeding-rats* were put in steel cages. The females were regularly checked for pregnancy. As soon as a female proved to be pregnant, it was isolated and placed in a special breeding battery.
   Food for these rats consisted of water and a standard diet (R.M.H., Hope Farms, Leiden, Netherlands) *ad libitum.*
3. *Age.* Determination of the time of conception by means of vaginal smears was omitted for practical reasons; we counted an animal's age from the moment of birth. Rats in which parturition might be expected, were checked three times a day.
4. Possible *sex-differences* in the various parts of the skeleton are at the age 4-11 days of negligible magnitude. Accordingly, the groups were made up irrespective of sex. But when an experiment was terminated, the animal's sex was always determined.
5. *Litter size* turned out to affect the growing young considerably. Experience taught us that litters of 8 rats gave practicable results. It was only by way of exception that we used litters of 7 or 9 rats. If litters were larger, on the 4^{th} day their number was reduced to 8. To prevent any disturbing effects, the young were taken from the nest as infrequently as possible.
6. *Formation of experimental and control groups* was done per litter. Suitable litters were assigned at random to either of these two groups. All rats in the litters of the experimental groups were operated upon. We were aware that this was not the most ideal procedure in theory. However, pilot studies - not described in this work - had shown that the presence of non-operated animals had an extra detrimental effect on the operated ones. Results were better if all the animals in one litter had received the same treatment. Neglect or cannibalism on the part of the mother would be very rare then.

B. OPERATIVE TECHNIQUES

1. *Operation outfit*
   The working surface was covered with operation drapes. The outfit consisted of:
   1. scalpels (Paragon Scalpel, Blade no. 11)
   2. dental instrument Ash 49
   3. small straight scissors
   4. surgical thread: Ethicon C 1 silk, type B-6-0
   5. small needle holder
   6. Switter pincettes

7. Petri disks with Ringer's solution (at room temperature)
8. rolls of cotton wool; pieces of gauze
9. Pasteur capillary pipettes
10. head lens (enlarging power 2,5 x)

The outfit was sterilized before use.

2. *Anaesthesia.* For anaesthesia ether was used (Aether anaesthesicus NP 35, Kon.Ned.Gist- en Spiritus Fabriek, Delft, Netherlands). For the purpose of administering anaesthesia the rats were put in a small square glass jar on a piece of metal gauze under which cotton wool saturated with ether had been placed. The jar was covered with a glass plate. After circa 4 minutes sufficient depth of anaesthesia was reached. In some animals respiration would stop, but as a rule this could be restored by artificial respiration with the help of a small air bulb. This way of anaesthetizing yielded good results. After some practice none of the animals died under anaesthesia.
3. *Assistance* during the operations was given by a trained biotechnician. This reduced the time of the interventions, especially the period that the implants remained in the antemedium, to 1-2 minutes.
4. *Numbering of the animals* was done following upon the operation by toe and/or finger amputation. In the implantation experiments with the third metacarpal bone only toes were clipped (Reitsma, 1963).
5. *Reports.* The operational proceedings and the findings on inspection (twice a day) were recorded.

C. OPERATIVE INTERVENTIONS

1. *Preparing costal cartilage implants.* With homologous implantations the litter-donors were decapitated under anaesthesia. After an incision parallel to the ribs had been made, the skin was retracted. The thin layers of muscle were removed, and the required costal sections set free and immersed in Ringer's solution.
   In obliquely incident light we can distinguish the epiphyseal cartilage from the other, costal cartilage owing to a difference in brilliance. This makes fairly accurate excision of the required cartilage fragments possible.
   With autologous implantation of costal cartilage, after one incision of about 6 mm a single costal section was cut loose and the above procedure followed. Frequently a lung collapse occurred. After suturing the wound a vacuum was again obtained by suction with an empty syringe, enabling the pneumothorax to recover.

2. *Preparing implants of the left-sided third metacarpal bone.* An incision of about 5 mm was made in the dorsal skin of the left hand. After retracting the skin, tendons and muscles were set free with the Ash 49 and the bone disarticulated. Proximally, it was necessary to break the firm bonds at right and left with the other metacarpals. The distal epiphysis was easily freed. Next, the object was immersed in Ringer's solution, checked for damage, and implanted immediately afterwards. The hand wound was stitched. Bleedings did not occur when the use of sharp instruments was limited. The controls were obtained in the same way.
3. *Unilateral condylectomy and preparation of the mandibular condyle implants.* With the scalpel a dorso-ventral incision of 7 mm was made 2 mm before the external ear. Next, the head was taken between thumb and index-finger, in dorsal flexion the skin was somewhat retracted, and the mandible gently pushed backwards. With the thin end of the Ash 49 the muscular tissue and the covering fasciae were then retracted, the articular capsule opened, and the condyle set free. The condyle was cut off with the scissors, along the visible junction of cartilage and bone. In this phase of the operation bleedings might occur, which, however, were rapidly stanched by slight pressure and suturing the wound (Figs. 2.1 and 2.2).
   After the condyle had been soaked in Ringer's solution, the articular disk, which as a rule would have come off too, was removed with the Ash 49 and a Switter pincette. Immediately afterwards the implant was put in position.
4. *Intracerebral implantation.* A median incision of about 7 mm was made with the scalpel across the cranial vault, and the skin retracted with the fingers. A square of about 2 mm was pricked into the middle of the left-sided parietal bone. Next, the pricked section was folded back. With a Pasteur capillary pipette the implant was sucked from the Ringer's solution, brought into the opening, and pressed into the cerebral matter to a depth of about 2 mm. The pipette had to be kept vertical to prevent the loss of the implant in the subdural cavity. The bone fragment that had been folded back, was replaced and the wound closed with a few stitches (Figs. 2.3 and 2.4).
5. *Subcutaneous implantation.* A 2 mm cut was made in the skin of the abdomen. With the Ash 49 a subcutaneous pocket was prepared. The implant was inserted with the Pasteur pipette and the wound closed in the usual way.
6. *Termination of experimental period.* Under ether anaesthesia the animals were decapitated. The implant was located and set free. After

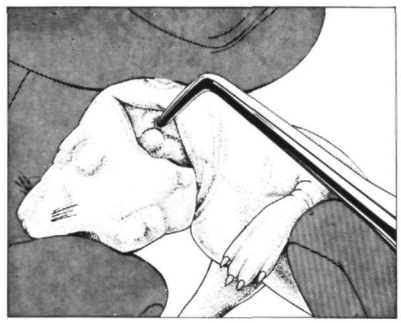

Figure 2.1

Figure 2.2

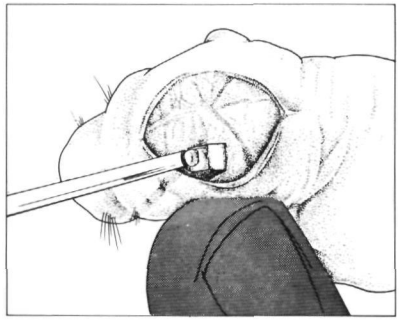

Figure 2.3

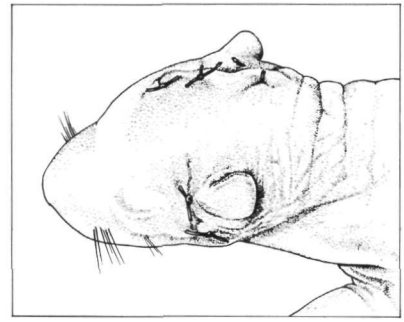

Figure 2.4

Figure 2.1
Unilateral condylectomy in 4-day rat
A dorso-ventral incision of 7 mm is made 2 mm before the external ear. With the thin end of the Ash 49 the muscular tissue and the covering fasciae are retracted, the articular capsule opened, and the condyle set free.

Figure 2.2
The condyle is cut off with the scissors along the visible junction of cartilage and bone.

Figure 2.3
A square of about 2 millimetres is pricked into the middle of the left-sided parietal bone. The pricked section is then folded back. With a Pasteur capillary pipette the implant is sucked from the Ringer's solution, brought into the opening, and pressed into the cerebral matter to a depth of about 2 mm.

Figure 2.4
The bone fragment that has been folded back, is replaced and the wound closed with a few stitches.

macroscopical inspection it was fixed. In the group which had the left-sided third metacarpal bone implanted, after termination of the experimental period the right-sided bone was set free also. In the group

where condylar implantation had been performed, the left and right mandibles were dissected out after fixation.

7. *Controls.* All control material was prepared and fixed in the same way as the material of the experimental groups.

D. PROCESSING THE MATERIAL

1. *Tracing method; measuring method.* On the greater part of the material - preparatory to histological processing - measurements were carried out. For the purpose of making tracings the preparation was put on a slide and any superfluous fluid removed with filter paper. By means of a Leitz Aristophot-apparatus with copying mirror the outline of the specimen was 12 x enlarged and reproduced on white drawing paper.

   3 Successive tracings were made of the largest shadow. This procedure was repeated twice at other times, so that altogether 3 x 3 = 9 drawings of the same specimen were obtained.

   The procedure was carried out three times in order to minimize the 'outline error'. Each outline was drawn three times to reduce the 'drawing error'. On the drawings every dimension was measured only once with a Mauser vernier gauge, to the nearest 0,1 mm.

   The maximum difference between the 9 measurements of a single object - apart from a few exceptions - did not exceed 1,5 mm on the drawings.

2. *Statistics.* Statistical processing of the observations was carried out by the Institute for Mathematical Service (Head: Drs. Ph.van Elteren), University of Nijmegen.

   For a comparison of mean values for each group the following tests were applied:

   a. Student's test for two samples, if the preliminary F-test had not shown any significant difference between the variances of observations for two different groups of rats. If the F-test did indicate a significant difference, it was followed up by Welch's test.
   b. Student's test for paired samples was applied if the observations had been carried out in pairs on the rats within one same group.

   The tests were always applied twosidedly.

   All figures indicating mean values and standard deviations are presented 12 x enlarged, because they derive from values measured on the drawings. Of the 9 measurements of each dimension the mean value was taken.

A difference found is called significant at the 5% level if its p-value is smaller than or equal to 0.05.

3. *Histological techniques.* 4% Formalin was the standard fixative, unless otherwise indicated. The paraffin sections (7 - 10 μ) were stained with:
   1. haematoxylin/eosin (Mayer);
   2. Toluidine Blue, 0.05% in H_2O;
   3. trichrome (Goldner);
   4. van Gieson.

   Tissues were processed according to the methods as described in the handbooks of B.Romeis (1948), H.Adam and G.Czihak (1964), and the laboratory guide by P.H.M. Schillings and J.C. de Haan (1964). An exception was made for the costal cartilage implants and the controls. After 24-hrs decalcification in 5% TCA in H_2O these were embedded in OCT (Lab-Tek, Westmont, Illinois) and cut into sections on a freezing microtome. The sections were coloured with Toluidine Blue and trichome.

E. SPECIAL TECHNIQUES

1. *Vital staining with tetracycline*
   Tetracycline (Achromycin)*) was used in the experiments with condylar implants to mark calcifying cartilage prior to implantation (Chapter 6).
   1. Injections
      The animals were given a single intraperitoneal injection, 24 hrs preceding the operation. The dose was about 0.1 mg per rat.
   2. Preparation and study of sections
      After fixation in 70% alcohol the undecalcified tissues were soaked in tetrahydrofuran (2 x 30 min.), embedded in paraffin (Tissuemat, 61°), and cut on the Leitz sledge-microtome. Sections at 5-7 μ were then mounted unstained and studied under the fluorescence microscope. A few representative sections were stained by the von Kossa method to localize the calciumphosphate.
2. *Tritiated thymidine autoradiography*
   Tritiated thymidine (^{3}H-TdR) was used to investigate mitotic activity in condylar implants and controls (Chapter 7).

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* This drug was kindly provided by R.I.T.-Nederland N.V., the Hague.

1. Injections
   Each animal operated on and carrying an intracerebral condylar implant, was given a single intracardial dose of about 50 μc. The specific activity of the ^{3}H-TdR was 1.9 c/mM.
2. Preparation of autoradiograms
   After terminating the test period, the implants and the right-sided condyle of the same animal were fixed in Bouin's fluid, decalcified in TCA 5%, embedded in paraffin (Tissuemat, 61°) and cut into sections at 5-7 μ.
   Autoradiograms were prepared, using Pelc's (1956) stripping-film technique, with Kodak AR-10 photographic plates. Exposure time was 14 days. The sections were then stained with Toluidine Blue 0.05% in buffer pH 4.

Chapter 3

TESTING THE *IN VIVO* IMPLANTATION METHOD

A. INTRODUCTORY

Experiments in which the *in vivo* implantation technique is used, should be interpreted with caution. In order to get a better idea of the usefulness of this method for our investigation we carried out a number of experiments that might be considered as a test of our specific experimental conditions. From these primary findings we have attempted, with the means at our disposal, to arrive at the best possible experimental design. Experimental conditions are mainly determined by the available animals, operative techniques, and the sites chosen for implantation.

With the *in vivo* implantation the host/donor relationship is of prime importance. One of its major aspects - at least in an investigation like the present one - is the prevention, whenever possible, of immunological rejection. This may be sought in three ways: First, by using an inbred strain (isologous or intrastrain implantation). The rat-strain at our disposal had been inbred only partially, and insufficiently for our purpose. Secondly, by homologous implantation between litter-mates. (Various workers have reported good results with this method, especially when young animals are used). A third method, which from an immunological point of view offers the best prospects, is that of autologous (intra-individual) implantation. It has the drawback of putting a heavier strain on the animal.

The feasibility of the second and third method was further tested by means of epiphyseal costal cartilage implants. These experiments also yielded information about the ways in which various histological processes were affected by them. Moreover, we got some idea about the variation in the behaviour of the implants and its possible causes.

B. THE BRAIN AS A SITE OF IMPLANTATION

From the literature it is clear that a great many sites allow of a more or less successful implantation of skeletal tissues. According to Felts, (1961) in the process of implantation the fundamental organo-genetic reaction of skeletal tissues is independent of the site where the implant is introduced. In choosing such a site, the nutritional possibilities for the implants are an important factor. After a brief avascular period, immediately after implantation, there is, as a rule, revascularization. The area most widely used is the subcutaneous one. Other sites are intramuscular, in the abdominal cavity, below the renal capsule, and intralienal. Additionally, the anterior chamber of the eye and the intracerebral area are two sites where an immunological reaction against an implant is thought not to occur, or at least much less readily. For the brain this phenomenon has been called 'the brain barrier'. There are other arguments that speak in favour of the brain as implantation site. Willis (1936), Moss (1957), and others with them, preferred the brain for their experiments with skeletal elements because subsequently there were excellent differentation, growth, and revascularization of the implants. Felts (1961), too, holds that in the brain revascularization is probably more intense and more rapid than in other sites. Crouse (1956, 1959, 1962) tested by means of various tissues (among others, embryonic limb buds) the suitability of the cerebrum as implantation site in the rat. He concluded that this area is very expedient for skeletal tissue implantation. Apart from advanced morpho- and histo-differentiation he, too, reported excellent revascularization. He found no or hardly any neurological disturbances.

Our own pilotstudies, not further referred to, also showed the formation of a connective-tissue capsule enveloping the implants. From sections of cerebral tissue with the implant *in situ* it is evident that this capsule is only a few cells thick.
Around the implants introduced subcutaneously for comparative examination, a thick layer of connective tissue was formed. Moreover, at the termination of the experimental period the intracerebral implants were much easier isolated than the subcutaneous ones. This is one of the reasons why intracerebral implantation presents better conditions for carrying out linear measurements. As main factor, however, must be seen the difference in the thickness of the capsule. A thin capsule enables us in histological sectioning to have good control of the plane of section of the cuts, which is conductive to an efficient comparison of the controls.

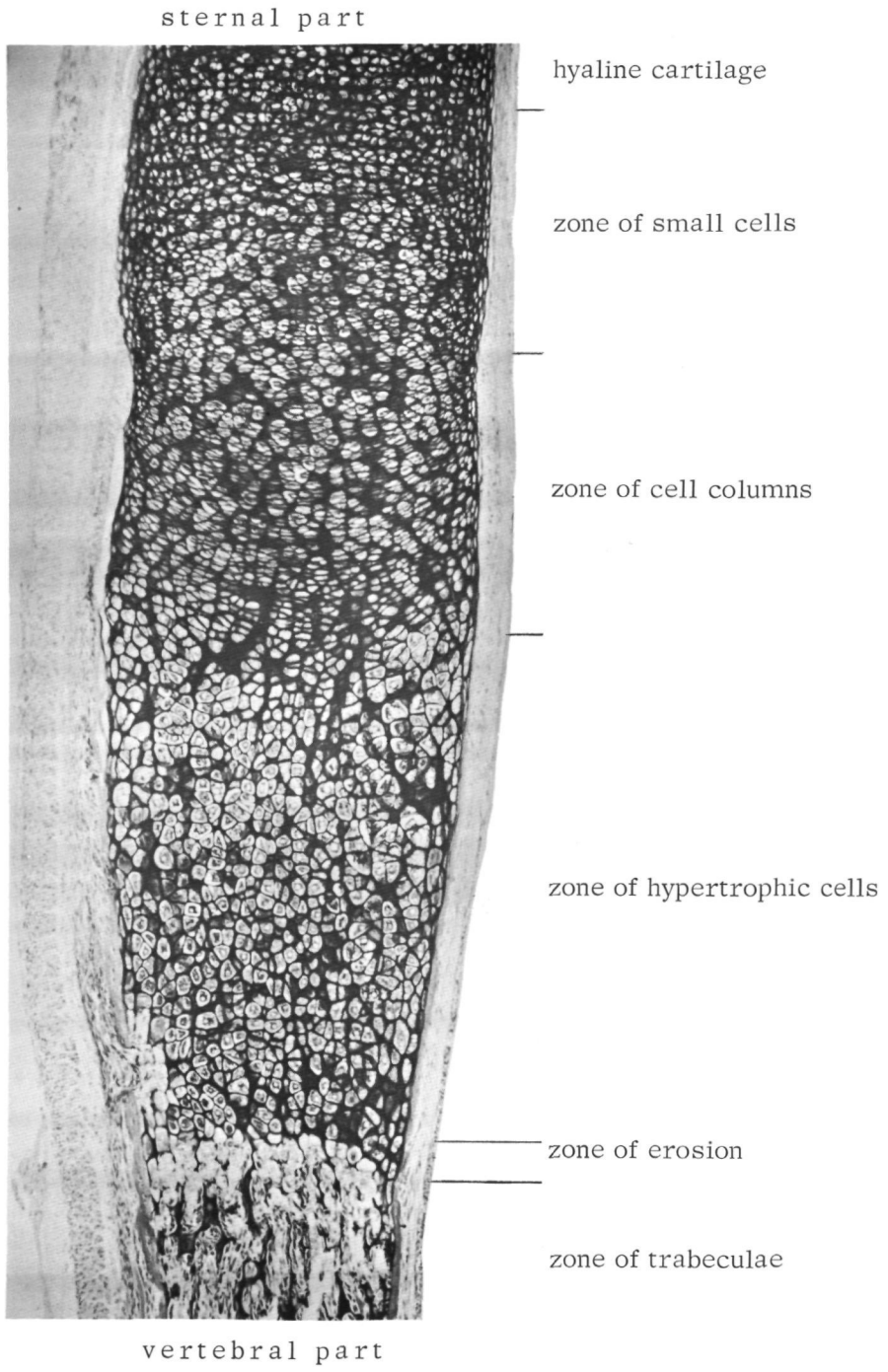
The intracerebral site has its drawbacks, too. The damage inflicted on the animal is likely to be more serious than in the case of subcutaneous or intramuscular implantation. We may also expect greater variation in revascularization, because introducing the implants at exactly the same place in the various animals is a more difficult task where the brain is concerned than for the other areas.

C. INTRACEREBRAL IMPLANTATION OF COSTAL CARTILAGE

1. *Epiphyseal cartilage*

The ribs of the 4-day rat may be distinguished in two parts of nearly equal length. The sternal part consists of cartilage, the other part of bone. At the junction of both there is epiphyseal cartilage. In this epiphyseal cartilage a number of zones may be discerned. Each zone contains cells at a certain stage of development, with a specific morphology and function. It is especially for the sake of an unambiguous terminology that these zones are mentioned here (Figs. 3.1 and 3.2). The sternal part consists of hyaline cartilage. This is succeeded towards the ossified vertebral part by a zone of small cartilaginous cells. Next comes the zone of cell columns, which gradually merges into that of the hypertrophic cells. This is joined by the zone of erosion and trabecular zone, which latter ends in the marrow cavity.

It is commonly assumed that in the zone of the cell columns there is interstitial growth of the cartilage as a result of frequent mitoses of chondrocytes and the formation of a cartilaginous matrix (Ham,1965; Weinmann and Sicher, 1955; Kember, 1960; and many others). On a basis of *in vivo* and *in vitro* experiments Holtrop (1964) holds that the zone of the small cartilaginous cells has a stimulating effect on this process. The older cells in the zone of the cell columns are gradually becoming hypertrophied. The matrix calcifies and the cells are broken down. Of the cartilaginous matrix, calcified trabeculae remain in existence, on which bone is deposited by the osteoblasts. Various interpretations of the course and nature of the processes in such an area of endochondral ossification will be discussed in Chapter 5(5.B).
Bone formation likewise originates from the perichondrium surrounding the hypertrophying cartilage; this bone formation goes side by side with an elongation of the bone shaft. The relationship of the perichondrium towards the whole is changed: the perichondrium turns into periosteum.


sternal part
hyaline cartilage
zone of small cells
zone of cell columns
zone of hypertrophic cells
zone of erosion
zone of trabeculae
vertebral part

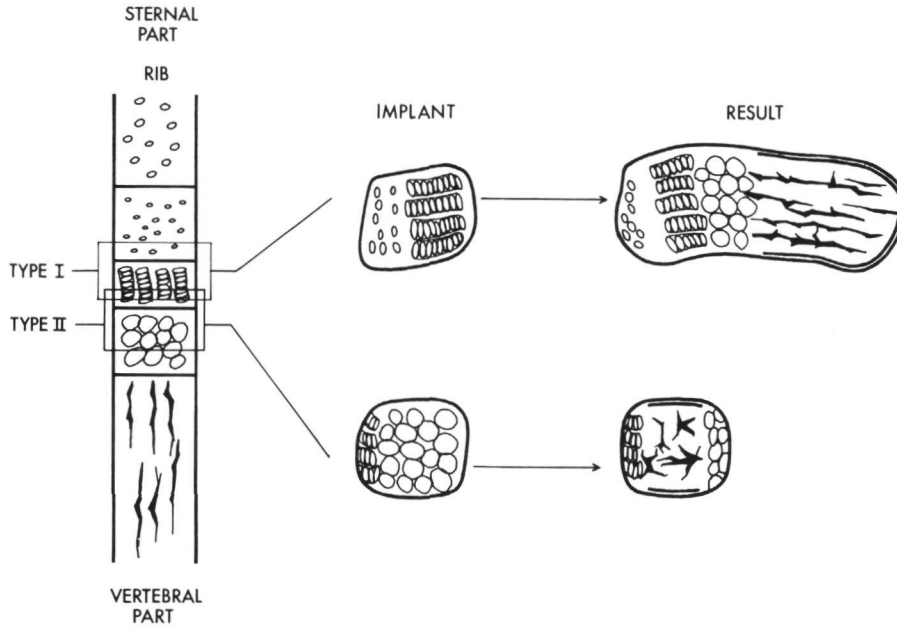


Figure 3.2
Diagram of performed intracerebral implantations of two different types (I and II) of fragments of epiphyseal costal cartilage
Result after 12 days.

Under normal circumstances during the period of growth the production and the break-down of chondrocytes are in equilibrium. This results in a longitudinal growth of the ossified part, based on the interstitial growth of the cartilage. In this respect it should be noted that we have here not so much a migration of cells as a migration of zones.

2. *Experimental design*

For these experiments 7 litters of 4-day rats were used. According to the part implanted, the experiment may be subdivided into:

Part A: Homologous implantations of type I. These were only performed on littermates.

Part B: Homologous implantations of type II, which besides on littermates were also performed autologously.

◄ *Figure 3.1*
Longitudinal section through the epiphyseal cartilage in the rib of a 4-day rat
The junctions of the zones are indicated approximately Bouin's fixative. H.E.; Magnification: x 118.

The choice of the types was analogous to that of one of Holtrop's experiments (1964). Type I implants contain small cartilage cells, and the zone of cell columns. Type II implants contain a fragment of the zone of cell columns and a considerable part of the zone of the hypertrophying cells. For a diagram see Fig. 3.2. Both types remained implanted in the cerebrum for 12 days.

When dissecting free the fragments of about 1 mm in length, we were unable to discern macroscopically whether the fragments did indeed contain the relevant part. Random selection of controls was therefore necessary. All required fragments were dissected free, soaked in Ringer's solution and stirred. Next, part of them were implanted and the remainder immediately fixed.
For a description of the operative interventions we refer to Chapter 2(2.C.1, 2.C.4, 2.C.6). The material, too, was processed as indicated there (2.D.1, 2.D.2, 2.D.3).

3. *Findings*

Findings Part A

Part A of the experiment comprised 2 groups:

Group A.1: the experimental group, consisting of 21 rats originating from 3 litters, on which homologous implantation of type I fragments from littermate-donors was performed.

Group A.2: consisted of 18 type I control fragments from the same donors.

As indicated above for both groups the selection of fragments was done at random.
20 Animals survived the experiment in good condition; all implants were retrieved. 7 Fragments turned out to be at almost right angles with the cranial vault, without, however, having become attached to it. Macroscopically, 19 out of the 20 fragments had increased considerably in length. In 18 fragments out of these 19 a transition from cartilage to bone, comparable to that of a rib *in situ*, was discernible.
The mean length of the controls was 14,28* (SD 2.56); the mean length of the implants was 31.64* (SD 6.65). The difference was so large that we refrained from a statistical test.

* The dimensions presented here (in millimetres) are those as measured on the tracings, and for this reason 12 x the actual dimensions of our specimens (see Ch. 2.D.2)

From all fragments sections were made in order to examine their histological structure. Out of the 18 controls 13 met our requirements. The other 5 in addition contained vestiges of the hypertrophying zone (cf. Table 3 A). The perichondrium was present around all fragments, except at the cut faces.

Table 3 A

Histological composition of tissue fragments used in Part A

Composition of type I implants after 12 days (Group A.1)		Composition of type I controls (Group A.2)	
entirely ossified	2		
small cartilage cells + columnar zone + hypertrophic zone + ossified part	12	small cartilage cells + columnar zone	13
idem + hypertrophic zone at end of ossified part	6	idem + part of hypertrophic zone	5
Total	20	Total	18

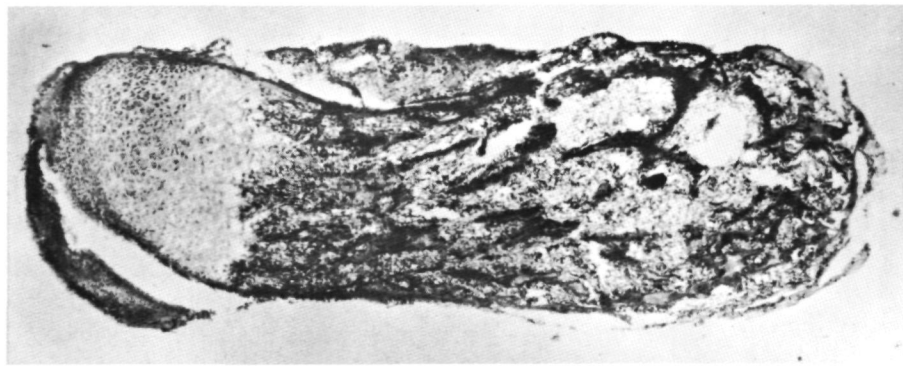


Figure 3.3
Type I fragment, 12 days after homologous intracerebral implantation on the 4th day in a littermate-rat

The zones normally occurring in costal epiphyseal cartilage *in situ* are again present. The processes of chondrogenesis, longitudinal growth and endochondral ossification have begun again.
Trichrome; Magnification: x 42.5.

Of the type I implants 2 were entirely ossified. One of them was the

implant that macroscopically had seemed not to have grown. The other consisted of an oblong piece of bone with a marrow cavity inside. All the 18 others presented zones normally occurring in the rib *in situ* (Fig. 3.3). In most cases the cartilaginous part dit not appear to have been reduced in size. The increase in length of the implants all but corresponded to the length of the bone fragment formed during the implantation period (Fig. 3.2).
In 6 of the implants we observed a phenomenon not seen in the others: at the end of the bony part there was a remnant of a narrow zone of hypertrophic cartilaginous cells. So in these implants two hypertrophic zones were visible, separated by a long bony cylinder containing trabeculae and a marrow cavity (Fig. 3.4). In the ossified parts of the implants small blood vessels were seen.

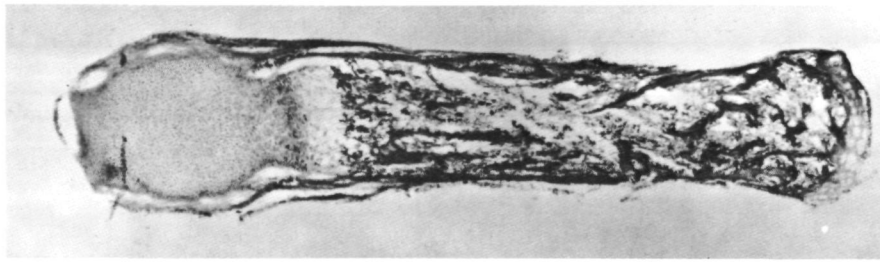


Figure 3.4
Type I fragment, 12 days after homologous intracerebral implantation on the 4th day in a littermate-rat
Note that at the end of the bony part of this implant there is a remnant of a narrow zone of hypertrophic cartilaginous cells. Thus in this implant two zones of hypertrophic cells are visible, separated by a long bony cylinder containing trabeculae and a marrow cavity. See also Figs.3.5; 4.7; 5.8.
Trichrome; Magnification: x 31.

Findings Part B
In this part of the experiment we worked with 3 groups:
Group B.1: this experimental group consisted of 15 rats, originating from 2 litters, on which animals homologous type II implantations from littermate-donors were performed;
Group B.2: consisting of 18 animals originating from 2 litters, on which autologous type II implantations were carried out;
Group B.3: consisting of 20 control fragments from the same donors as in group B.1, which fragments after dissecting out had been fixed.

For this part of the experiment, too, the fragments for groups B.1 and B.3 had been selected at random.

Of the 15 rats on which homologous implantations had been done (group B.1), 7 died after a few days. When the experiment was terminated, we failed to locate 2 implants. The 6 implants that were retrieved did not present any macroscopical changes of shape or structure.

The findings from the autologous implants were more successful. Out of the 18 B.2 rats, 15 survived in good condition. 3 Rats died on the day of operation. When the implants were located, one of them turned out to have become united with the cranial vault. All other fragments presented the same macroscopical picture as the retrieved homologous implants.

The mean length of the control fragments (group B.3) was 13.75 (SD 1.84). The retraced B.1 implants had a mean length of 13.75 (SD 2.83). After the experiment the mean length of the autologous implants (group B.2) was 13.65 (SD 1.27). The differences were so slight as to make a statistical examination unnecessary.

Table 3B

Histological composition of tissue fragments used in Part B

Composition of implants after 12 days (type II)				Composition of controls (type II)	
Group B.1		Group B.2		Group B.3	
homologous		*autologous*			
entirely ossified	2				
endochondral ossification, with remnants of cartilage at cut faces	4	endochondral ossification, with remnants of cartilage at cut faces	13	part of columnar zone + hypertrophic zone	9
				hypertrophic zone only	10
		no changes	1		
		unfit for assessment	1	unfit for assessment	1
Total	6	Total	15	Total	20

The results of the investigation into the histological structure of the fragments from the 3 groups are listed in Table 3.B. It can be seen that

setting free the control fragments has not been altogether successful, since only 9 out of the 19 fragments include part of the zone of cell columns. 1 Control fragment could not be judged because its processing went wrong.

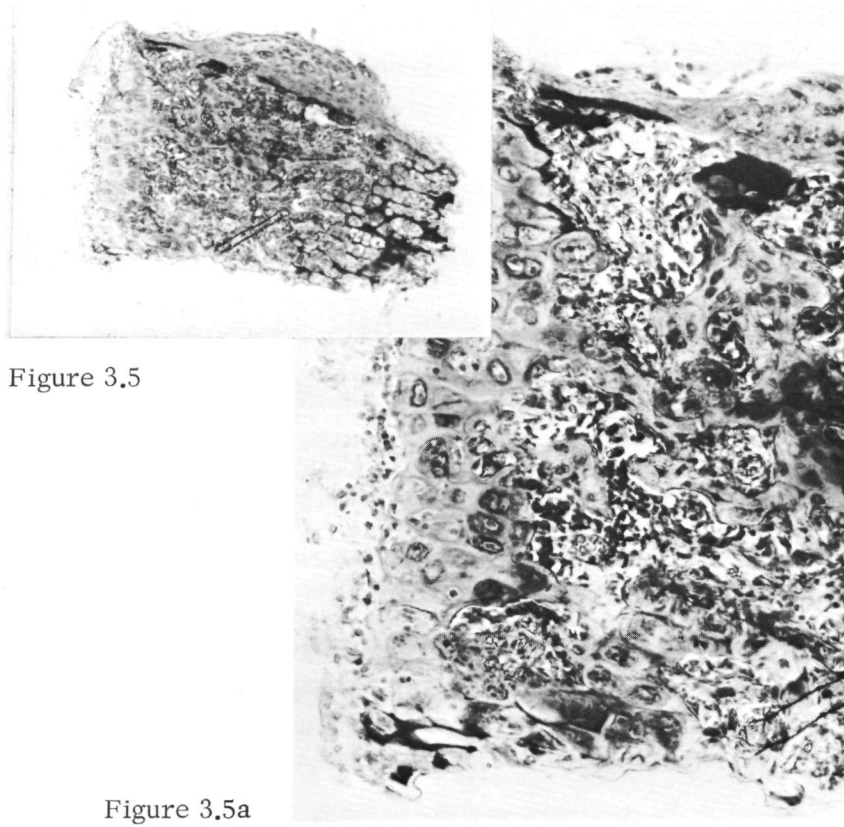


Figure 3.5

Figure 3.5a

Figure 3.5
Type II fragment, 12 days after autologous intracerebral implantation on the 4th day
There are cartilaginous remnants at the cut faces, while in the centre, endochondral ossification has begun. No chondrogenesis or longitudinal growth occurred. Note the bone formation at the outside of the perichondral tube.
Tol. Blue; Magnification: x 39.

Figure 3.5a
Higher enlargement of the left part of Fig.3.5
Tol. Blue; Magnification: x 127.

Of the 6 homologous implants retrieved (group B.1), 2 were shown to consist of bone, presenting a marrow cavity, bony trabeculae, connec-

tive tissue and bone marrow cells. In the other implants of the group, as well as in the autologous cases (group B.2), there were cartilaginous remains at the cut faces, while at the centre endochondral ossification had begun (Figs. 3.5 and 3.5a). It appeared as if small blood vessels and connective tissue cells had penetrated from the surrounding, co-implanted perichondrium. From the observations made of the controls (group B.3) it would seem that it hardly mattered whether part of the zone of cell columns was co-implanted or not.
In a single autologous implant (group B.2) we found bone formation at the outside of the perichondral tube. One implant of this group showed no visible changes. Here, too, one specimen could not be assessed owing to processing failure. All implants with the exception of the single one that showed no changes, were vascularized.

* * *

Table 3C
Chances of survival for operated animals
(for explanation see text p.**)

Groups	survived	died	Chances of survival
A_1 (homologous type I)	20	1	$A_1 > B_1$
B_1 (homologous type II)	6	7	p = 0.002
B_1 (homologous type II)	6	7	$B_2 > B_1$
B_2 (autologous type II)	15	3	p = 0.05
A_1 (homologous type I)	20	1	not significant
B_2 (autologous type II)	15	3	

> = significantly greater than

For the parts A and B of the experiment we also looked for any significant differences as regards the mortality rate between the groups of rats operated upon (Table 3C). Our criterion here was whether an animal survived the 12-day time-span of the experiment. Cannibalism or rejection of the off-spring did not occur in these experiments. When we failed to retrieve an implant this was interpreted as an artefact and the operation considered not to have taken place. Table 3 C shows that the chances of survival for rats having a homologous type I implant (group A.1) are significantly greater than for rats having a homologous type II implant (group B.1). The survival chances for rats with an autologous

type II implant (group B.2) are greater than those for rats with a homologous implant (group B.1).

D. DISCUSSION AND CONCLUSIONS

From the histological composition of the control group specimens could be concluded, that the setting free of the experimental fragments showed a larger variation than was anticipated. This holds true for type I as well as type II implants. We assume a comparable variation to have existed in the implanted material, which fact would go far to explain the variation in the histological picture of the implants in each of the 3 experimental groups.

The difference between the results of the type I and type II implants is remarkable. Obviously, the zone of cell columns, when accompanied by at least part of the zone containing the small cartilaginous cells, is capable of longitudinal growth, which was very evident with the type I implants. In both types the process of ossification had begun again. In the type II implants there was only ossification; no longitudinal growth was observed.

It is likely that in the 6 type I implants which showed a narrow hypertrophic zone at the end of the ossified part, this zone had already been present before the implants were introduced. Presumably varying according to the site of cutting concerned, the development of the cells at the cut face turned out to be disturbed, which phenomenon we propose to term the 'cut face effect'. It was found in nearly all type II implants. Among the factors that may be thought to have contributed to the effect are: the lesions caused to the cells at the cut face, the avascular period, and the stage of development of the cells. Of these the present author considers the avascular period the most important.

The break-down process of the hypertrophying cells is closely linked with intensive vascularization. If this link is severed, the cartilage cells do not die in due time nor does any ossification set in, or if it does, it is retarded. (Trueta and co-workers, 1960). In 12 type I implants no cut face effect was seen. Evidently, the experimental procedure and the avascular period were of less influence here. It is a well-known fact that the nutrition of cells in the zones as aimed at in the type I implants is more directly dependent on diffusion and only indirectly on vasculari-

zation.
Another factor could be the difference in cell age. Type I implants contain cells younger than type II ones. In the zone of cell columns, cells are found that have just been formed. The hypertrophying cells, on the other hand, are approaching the end of their life span. It is common knowledge that younger tissues are more capable of standing implantation procedures than older ones.

We also tried to find a plausible explanation for the origin of the ossicles. One ossicle (type I implant) showed, in fact, longitudinal growth. It is likely that here proliferation of cartilage had indeed occurred, but that this cartilaginous growth soon came to an end and was followed by general ossification. The other type I ossicle may have been wrongly prepared. The ossicles of the homologous implant group, type II, may owe their origin to a shorter avascular period or to more intense vascularization. But it is impossible to say what factors are responsible for the general ossification.

The experiments described here are best compared with Holtrop's (1964). She implanted costal cartilage fragments from 18-day embryo mice subcutaneously as well as intramuscularly, which contained about the same zones as our types I and II. She, too, observed longitudinal growth and endochondral ossification in implants like our type I. Her implants that were comparable to our type II, however, passed entirely into ossicles, without any increase in length. Presumably this difference has something to do with the age of the donors and the site of implantation. In some of these implants she also observed remnants of hypertrophic cartilage.

The significant differences found between the mortality rates of the homologous type I and II implants might be interpreted as a slight indication of certain differences between the two types of implants as regards their ability to induce an immunological reaction.
The difference in mortality found between the homologous and the autologous implants is even more significant if one considers that despite the extra strain of the thorax operation there were more survivors in the autologous group.

Chalmers and Ray (1959) were able to demonstrate a difference in effect of the homograft immune reaction between new-formed cartilage and bone. Presumably a similar phenomenon played a role here.

To sum up, we may say that in order to find out the best possible procedure we implanted - homologously as well as autologously - various fragments of epiphyseal costal cartilage. The brain was found to be a suitable site for implantation: after an avascular period an intensive revascularization of the implants would follow. On the basis of the experiments and findings as reported in the present chapter we decided to employ in subsequent experiments exclusively the method of autologous implantation. It was also thought appropriate to investigate how the epiphyseal cartilage reacts when the link with the neighbouring areas is not severed, as happens when an entire long bone is implanted.

Chapter 4

AUTOLOGOUS IMPLANTATION OF THE THIRD METACARPAL BONE

A. INTRODUCTORY

From the experiments concerning the implantation of costal cartilage, as described in the previous chapter, it was found that the various cartilaginous zones reacted differently to the intervention. Most evident was the retarded development of the hypertrophying cartilage at the cut faces. This raised the question in how far this process would also occur when the zone of hypertrophying cells would retain its link with the zone of the trabeculae and the marrow cavity. This problem was investigated by implanting a long bone *in toto*. For the purpose we chose the third metacarpal bone*, which had proven to be suitable in preliminary tests. Such an implantation will interrupt vascularization, which is especially intense in the marrow cavity and in the periosteum.
Furthermore, the experiment provided an opportunity for observing the changes at the articular surface. This was important here, because it yielded material for comparison with the behaviour of the articular surface of the implanted mandibular condyle.
The influence of implantation on size and shape was also studied. By extending the investigation to include the third metacarpal of the right hand (which bone had remained *in situ*), it was possible to get a general idea of the effects of the intervention.

B. EXPERIMENTAL DESIGN

In addition to the experimental group, 2 control groups were selected, to supply information on 4-day and 11-day metac.III. Starting from 9 litters 3 groups were formed:
Group 1: An experimental group of 3 litters, each of 8 rats. On all animals we performed an autologous, intracerebral implantation

* Hereafter abbreviated as: metac. III

of the left-sided metac.III on the 4th day. After 7 days the animals were killed. The implant, as well as the right-sided metac.III, was set free and fixed;

Group 2: A normal control group of 4-day-old rats, in which the left metac.III was freed and immediately fixed. In this way 20 good specimens were obtained;

Group 3: A normal control group of 11-day-old rats, in which the left metac.III was set free and immediately fixed. From this group 20 specimens became available.

Operations were carried out according to the methods described in Chapter 2 (2.C.2, 2.C.4, 2.C.7).

C. MEASUREMENTS

22 Animals survived the experiment in good condition, but in 2 of them the implants were revealed to have been placed subcutaneously instead of intracerebrally. These animals were discarded.

After fixation, measurements were carried out as described in Chapter 2 (2.D.1). The data obtained were subjected to statistical processing (2.D.2). 3 Dimensions were determined in each of the specimens. (Fig.4.1): 1. maximal length;

2. diaphyseal diameter;
3. epiphyseal diameter.

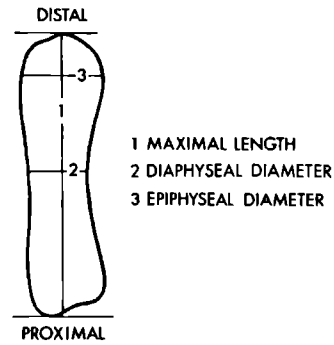


Figure 4.1
3 Dimensions were determined on each tracing of the metacarpals

In addition, the following three ratios were computed:

(1) $\frac{maximal\ length}{epiphyseal\ diameter}$ (2) $\frac{maximal\ length}{diaphyseal\ diameter}$ (3) $\frac{epiphyseal\ diameter}{diaphyseal\ diameter}$

The means and standard deviations obtained are presented in Table 4 A. The significance of the differences between the measurements and between the ratios are listed in Table 4 B. The findings are schematically illustrated in Figs. 4.2 and 4.3.

Table 4 A

Table of number (n), means (M), and standard deviations (SD) of measurements, and of ratios of these measurements, per group of IIIrd metacarpal bones *

		C4	C11	IM	R11
	n	20	20	20	20
maximal length	M	29.12	46.45	37.54	42.34
	SD	1.12	1.90	1.62	2.67
epiphyseal diameter	M	10.06	14.68	12.92	13.81
	SD	0.69	0.85	0.96	0.98
diaphyseal diameter	M	7.04	9.26	7.95	9.42
	SD	0.41	0.41	0.80	1.02
maximal length / epiphyseal diameter	M	2.91	3.17	2.92	3.07
	SD	0.22	0.12	0.22	0.13
maximal length / diaphyseal diameter	M	4.20	5.03	4.76	4.52
	SD	0.28	0.23	0.48	0.26
epiphyseal diameter / diaphyseal diameter	M	1.43	1.59	1.63	1.47
	SD	0.10	0.10	0.13	0.11

C 4: 4-day left IIIrd metacarpal (normal Control Group 2)
C11: 11-day left IIIrd metacarpal (normal Control Group 3)
IM : autologously implanted left IIIrd metacarpal (Experimental Group 1)
R11: right IIIrd metacarpal (Experimental Group 1)

* The dimensions listed here (in millimetres) are those as measured on the tracings, and for this reason 12x the actual dimensions of our specimens.

Metrical analysis confirmed that over the period studied a normal bone shows an absolute increase of all the dimensions investigated. At the same time the bone modifies its shape and becomes more slender, which process is most evident in the diaphysis. On comparing the normal

Table 4 B

Significance of differences between measurements and ratios of measurements of groups of IIIrd metacarpal bones, tested by Student's t test for two samples

	C11-C4	IM-C4	C11-IM	C11-R11	R11-C4
maximal length	+++	+++	+++	+++	+++
epiphyseal diameter	+++	+++	+++	++	+++
diaphyseal diameter	+++	+++	+++	N.S.	+++
$\frac{\textit{maximal length}}{\textit{epiphyseal diameter}}$	+++	N.S.	+	++	++
$\frac{\textit{maximal length}}{\textit{diaphyseal diameter}}$	+++	+++	+++	+++	+++
$\frac{\textit{epiphyseal diameter}}{\textit{diaphyseal diameter}}$	+++	+++	N.S.	+++	N.S.

\+ at the 5% level ($0.01 < p \leq 0.05$)
++ at the 1% level ($0.001 < p \leq 0.01$)
+++ at the 1‰ level ($p \leq 0.001$)
N.S. not significant

The measurement placed first is the larger of the two.
C 4: 4-day left IIIrd metacarpal (normal Control Group 2)
C11: 11-day left IIIrd metacarpal (normal Control Group 3)
IM : autologously implanted left IIIrd metacarpal (Experimental Group 1)
R11: right IIIrd metacarpal (Experimental Group 1)

4-day-bones (Group 2) with the implant group (Group 1), an enlargement of the dimensions was likewise found, be it of another order. But the modifications in shape were different: only in the diaphysis had the implants become more slender, not in the epiphysis. When the implants were compared with the normal 11-day control group, all the implant dimensions turned out to be smaller. The implants were also less slender, but the ratio between the epiphyseal diameter and the diaphyseal diameter did not show a significant difference from that of the normal controls of the same age. The right-sided metacs. III of the experimental groups, which had remained *in situ*, were shorter and presented a smaller epiphyseal diameter than the 11-day controls (Group 3). The dia-

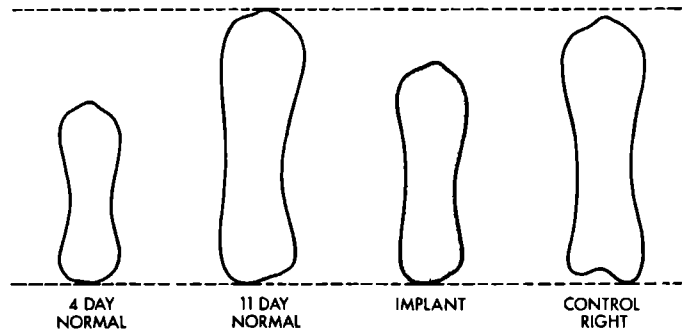


Figure 4.2

Diagram showing the differences in length of the 4 groups of metacarpal bones

The dimensions in this drawing are based on the mean length for each group. The implant (left 3rd metacarpal, 7 days after autologous intracerebral implantation on the 4th day) shows an absolute increase of the dimensions investigated. Note that the control right (3rd metacarpal from the experimental group 1 that remained *in situ*) is slightly shorter than the 11-day normal (group 3).

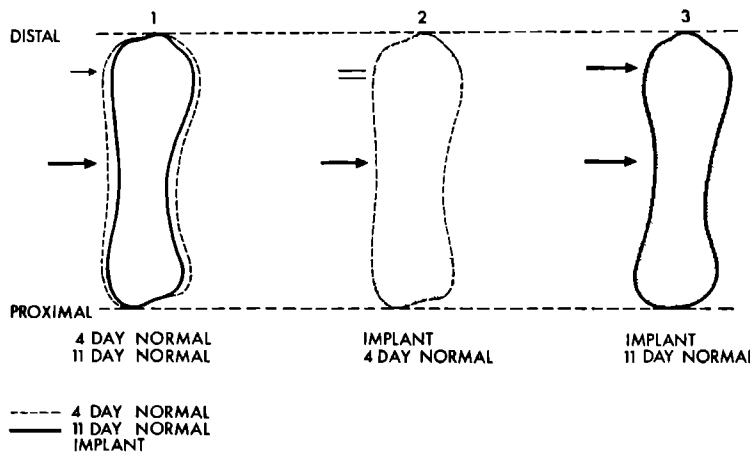


Figure 4.3

Diagram showing the differences in shape in proportion to length

The dimensions in this drawing are based on the mean length, the mean diaphyseal diameter and the mean epiphyseal diameter for each group of 3rd metacarpals. For illustrative purposes the proximal parts are drawn in, but they were not measured. The differences in changes between the epiphyseal and diaphyseal diameters have been accentuated by arrows.

physeal diameter did not differ significantly between the two groups. The entire complex of modifications resulted in a difference of shape. On comparing observations within the experimental group it was found that the length of the implanted metac.III was always appreciably less

than that of the right-sided bone of the same animal. The epiphyseal diameter of the implants was greater in 3 cases, the diaphyseal diameter in 2. These differences were very slight, however.

D. HISTOLOGICAL INVESTIGATION

From all the metacs.III of the 11-day specimens (groups 1 and 3) and from the left hand of two 4-day rats serial histological preparations were made (Figs. 4.4 and 4.4[a]).

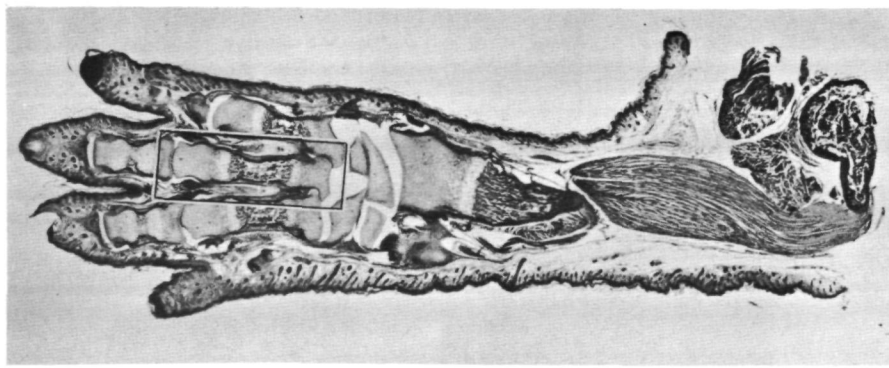


Figure 4.4

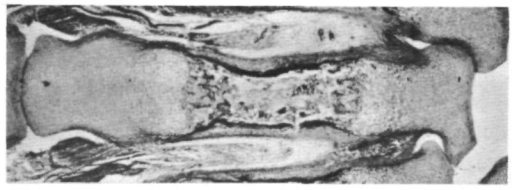
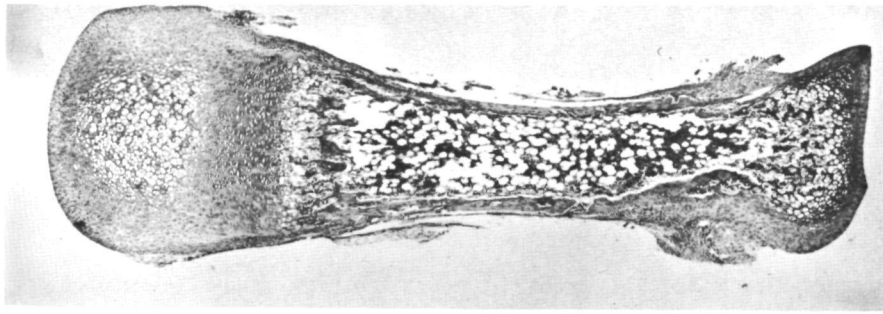


Figure 4.4a

Figure 4.4
Longitudinal section through the left hand of a 4-day rat, showing the stage of skeletal development
Inset: the 3rd metacarpal bone
Trichrome; Magnification: x 10.

Figure 4.4a
Higher enlargement of the inset in Fig.4.4, showing the 3rd metacarpal bone of a 4-day rat with a bony diaphysis with two cartilaginous epiphyses
Epiphyseal cartilage, endochondral ossification and marrow cavity are present. The diaphysis is enveloped in a thick cellular periosteum. Articular cavities are fully formed. No zone of articular cartilage can as yet be distinguished from the rest of the cartilage.
Trichrome; Magnification: x 27 (same enlargement as Figs.4.5 and 4.6).



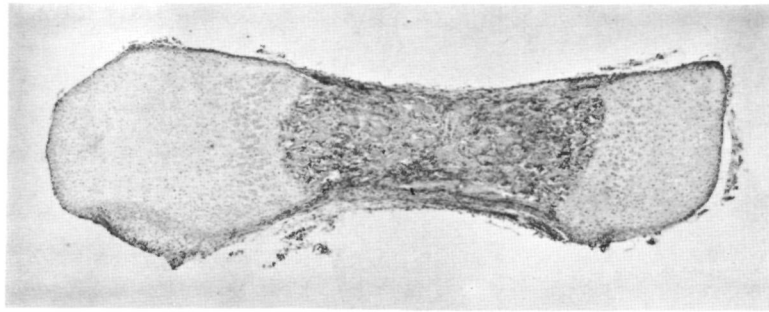


Figure 4.5
Longitudinal section through the 3rd metacarpal bone of a normal 11-day rat (control group 3)

The distal (leftside of the figure) epiphyseal disk is highly regular. At the centre of the distal epiphysis there is a focus of intensely hypertrophying chondrocytes. The articular cartilage is easily seen.
H.E.; Magnification: x 27 (same enlargement as Figs.4.4a and 4.6).

Figures 4.6
Longitudinal section through the 3rd metacarpal bone, 7 days after autologous intracerebral implantation on the 4th day (implant from experimental group 1)

No bloodvessels or connective-tissue strands are visible in the cartilage. Epiphyseal zones are narrower. The shape of the diaphysis is irregular, with thick bony trabeculae protruding into the marrow cavity. See also Figs.4.4a; 4.5; 5.11.
H.E. Magnification: x 27 (same enlargement as Figs.4.4a and 4.5).

In the 4-day metac.III a bony diaphysis with two cartilaginous epiphyses was found. The epiphyseal cartilage, endochondral ossification and marrow cavity were already present. The diaphysis was enveloped in a thick cellular periosteum. The articular cavities had been fully formed.

No zone of articular cartilage could as yet be distinguished from the rest of the cartilage.

Histological examination of Group 3 yielded the following data (Fig. 4.5). In the 11-day metac.III, ossification has already made considerably more progress. The epiphyses are relatively smaller. Distally, at the centre of the epiphysis, there is a focus of intensely hypertrophying chondrocytes. In 11-day specimens the articular cartilage is easily seen. Here the cells are fairly small and from ovoid to spheroid. The epiphyseal disk is highly regular. The hypertrophying and trabecular zones are narrow. The diaphysis consists of a fairly thick and compact cylinder of bone. Besides numerous blood vessels, the marrow cavity contains haemopoietic tissue. Osteoclasts occur especially at the ends of the trabeculae.

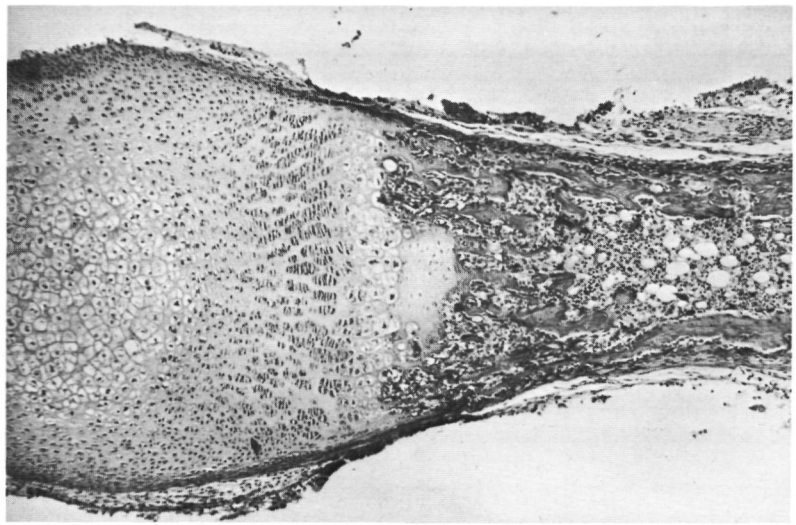


Figure 4.7
Longitudinal section through the distal part of a 3rd metacarpal bone, 7 days after autologous intracerebral implantation on the 4th day (implant from experimental group 1)
The zone of hypertrophying cells is damaged: a cluster of cartilaginous cells has failed to be eroded in time.
H.E.; Magnification: x 66.

Histologically, no differences could be found between the 11-day controls (group 3) and the metacs. III of group 1 that had remained *in situ*.

A comparison of the implants with the 11-day controls showed quite another state of affairs (Fig. 4.6). True, in the same way as with the controls, in the distal epiphysis of the implants a focus of intensely hypertrophying chondrocytes was observed. In the implants, however, the articular zone was narrower and less manifest. It was especially there that remnants of the thin capsule of connective tissue were visible which had formed around the implants. Nowhere had blood vessels or connective tissue strands penetrated into the cartilage. The epiphyseal zones were narrower. The trabecular zone, on the other hand, had widened considerably. In several implants the zone of hypertrophying cells was damaged: a cluster of cartilaginous cells had failed to be eroded in time (Fig. 4.7). In such a case the matrix would be achromatic. Osteoclasts were seen, but their occurrence was not so regular as in the controls. The shape of the diaphysis was irregular, with thick bony trabeculae protruding into the marrow cavity. This cavity was smaller; it contained sparse haemopoietic tissue, but numerous connective tissue elements and blood vessels.

E. DISCUSSION AND CONCLUSIONS

Measurements did show growth of the implanted specimens, but they lagged behind the controls.
Changes in shape, too, were different. From metrical analysis it might be inferred that over the experimental period there was no difference in reaction to the implantation as far as the epiphyseal and diaphyseal diameters were concerned. Hence the other pattern of growth might be thought to have its origin in a more pronounced retardation in longitudinal growth. In other words: the relatively too large size of the cartilaginous head of the implants over this period was not due to deviant differences between the increasing diameters of epiphysis and diaphysis, but to a deficiency in longitudinal growth. The histological picture did not reveal whether this resulted from a lesser degree of interstitial growth within the epiphyseal cartilage; the epiphyseal zones were a good deal thinner, however.
Felts et al. (1961) worked with isologously and subcutaneously implanted neonatal mouse humeri and rat phalanges. They hold the view that the early re-establishment of nutrition of the surface cells will first cause an increase in diameter; longitudinal growth commencing at a later stage. The hypertrophying cartilaginous zone of the implanted metac.III reacted in a different way on the implant procedure than the costal car-

tilage implants. In the implanted metac III, the erosion of the hypertrophying chondrocytes had generally continued. This may be due to the fact that not an abrupt but a more gradual interruption or delay of the indispensable nutritional substances had occurred.

In a number of implants we saw a disturbance in the endochondral ossification in the form of an achromatic cartilage islet. Felts thinks the presence of the shaft to be a barrier to a rapid restoration of adequate nutrition.

The metaphyseal trabeculae were appreciably elongated and had evidently not been resorbed in due time. The fact that on the endosteal side of the diaphysis the resorption processes had become seriously disturbed was obvious from the presence of bony trabeculae penetrating into the marrow cavity. They are presumably bony remnants of the original diaphysis. The deposition, too, from the periosteum, which latter is very dependent on an intensive vascularization, was probably retarded over some period of its growth. A study of the articular cartilage showed that it does undergo some development, even if it is more retarded and thinner than normal. In Felt's long-term experiments, however, the articular cartilage disappeared entirely.

Comparison of the right-sided metac.III that had remained *in situ*, with the normal controls showed that the operated animals were lagging in skeletal growth. There was no clear indication that they were also lagging in skeletal maturation, as determined by the development of the secondary ossification centre in the distal epiphysis. The results from this experiment are in agreement with the findings of Felts (1959) obtained from isologous subcutaneously implanted rat and mouse humeri.

Worthy of mention here is another experiment by Felts (1961), in which he separated the distal and proximal cartilaginous parts from the diaphysis in 2-day mouse humeri. The extent of growth in a 28-day implant of the separate parts was, taken together, as least as much as if they had been implanted as a whole. The diaphysis, however, had not lengthened at all.

Chapter 5

AUTOLOGOUS IMPLANTATION OF THE MANDIBULAR CONDYLE

A. INTRODUCTORY

The experiments as described in Chapters 3 and 4 have shown that both isolated epiphyseal cartilage and entire long bones, once implanted, are capable of remarkable development. Similar results were also obtained, by other workers from other cartilaginous parts of the skeleton, other sites of implantation, and other species. There is, indeed consensus that chondrogenesis as well as endochondral ossification are largely independent of immediately environmental factors.

Considering the entirely different phylogenesis and ontogenesis of the mandible and the mandibular joint, our next problem would seem to be the question of whether chondrogenesis and endochondral ossification - the first of which is mainly responsible for the increase in length of the ascending ramus - have a comparable autonomy.

For further investigation into this matter a few experiments were carried out. In a number of rats the mandibular condyle was isolated and subsequently implanted autologously into the cerebrum. Here, too, the implants were examined for any dimensional and histological changes. In all measurements, as well as in the histological examination, only one plane was considered, viz. the one in which the major part of the relevant mandible was situated. It is in this direction that the growth of the mandible is most pronounced. The plane is parasagittal but is not quite parallel to the median plane.
The experiments of Chapter 3 had shown that the presence of cells from a zone unintentionally implanted might be considered a major factor in the behavioural variation of the costal cartilage implants. Hence it is of extreme importance that the cartilage should be cut at a spot that can be precisely determined macroscopically. In Chapter 2 it was mentioned that in a 4-day rat the junction of the condylar cartilage with the bone of the ascending ramus is fairly smooth. The junctions with the

other zones in the direction of the articular surface are arched. Furthermore from Chapter 3 it will be clear that cells of varying maturity, like proliferating and hypertrophying chondrocytes, react differently to implantation. On the basis of these considerations it was assumed that the best place to apply a cut was in the hypertrophying cartilage, just above the zone of erosion. Thus one obtains a cut face that presents a maximum of cartilaginous cells of equal maturity. Moreover, such a site allows of good macroscopic determination.

* * *

Before describing the experiments it seems appropriate to give a brief survey of the morphogenesis and histological structure of the mandibular condyle.

B. MORPHOGENESIS OF THE MANDIBULAR CONDYLE

The data in the literature on this subject are inadequate. In order to get a better insight into the genesis of the mandible of the rat we carried out a systematic investigation, comprising a period from 14 days after conception till 14 days after birth. Besides the usual histological techniques we also employed the vital staining method. The data mentioned below derive mainly from this investigation.*

In the embryonic rat on the 14th day ***post conceptionem***** a bony plate develops laterally and at some distance from the previously formed cartilaginous bar which is known as Meckel's cartilage. This bony plate is formed within a mesenchymal membrane rich in cells. At the caudal end of this membrane on the 16thday p.c. a blastema is formed, which has two cell condensations. The superior one constitutes the anlage of the condylar cartilage, the inferior one the anlage of the angular cartilage. Both assume a carrot-like shape. Subsequently these cartilaginous 'carrots' are partly enclosed by the growing bone, as a result of which a perichondral bony tube is formed. This bony tube is lined with a thick periosteum, rich in cells, which towards the end passes into a thick layer of chondrogenic cells covering the cartilage. On the 18th day p.c. the first articular fissure appears; the anlage of the articular disk can now be discerned.

* The investigation mentioned here into the morphogenesis of the mandible of the rat will be published elsewhere.

** Hereafter referred to as p.c.

On the 20th day p.c., which is as a rule the day before birth, the second articular fissure is formed, entirely separated from the first fissure by the articular disk. The condylar cartilaginous carrot gradually disappears, largely owing to endochondral ossification. 4 Days after birth it is only the round condyle which still consists of cartilage; it is now enclosed by a narrow strip of osseous tissue. At the same time the layers superimposed upon the cartilage are more and more prominent. The histological structure may be easily understood from the situation at 9 days after birth. At this time we can distinguish 6 layers or zones (Figs. 5.1 and 5.1a):

1. articular layer;
2. proliferating layer;
3. transitional layer;
4. zone of cartilage;
5. zone of erosion;
6. zone of cartilaginous trabeculae.

The articular layer is adjacent to the articular cavity and consists of two parts: a cellular part, situated at the surface, which constitutes the lining of the articular cavity; and underneath this a second one, fibrous and much less rich in cells. This second part is of even thickness throughout; the cells lying in it are small and flat, and have their greatest dimension parallel to the articular surface. The proliferating layer, coming next, is readily distinguished from the articular layer. Here the cells are considerably larger and rounder, and relatively speaking there is much less intercellular matter. The proliferating layer is approximately 5-8 cells thick and displays mitotic figures. Our investigation showed that the cells divide with their spindle axes parallel to the articular layer, as may be clearly seen from Fig. 5.2. This observation is in agreement with Kember's (1960) findings for the direction of cell division in epiphyseal cartilage. The layer is thickest in the distal part of the condyle and tapers off towards the periosteum. In our own experiments with tritiated thymidine, as described in Chapter 7, two hours after injection a large number of labelled cells were seen almost exclusively in the proliferating layer, which fact indicates that many cells in this layer are preparing for division. This conforms to the findings from experiments with tritiated thymidine by Dale et al. (1963) and Blackwood (1966).

The proliferating layer is not made up of cartilage. For the cells of this layer several names have been suggested (Öberg, 1964). We prefer

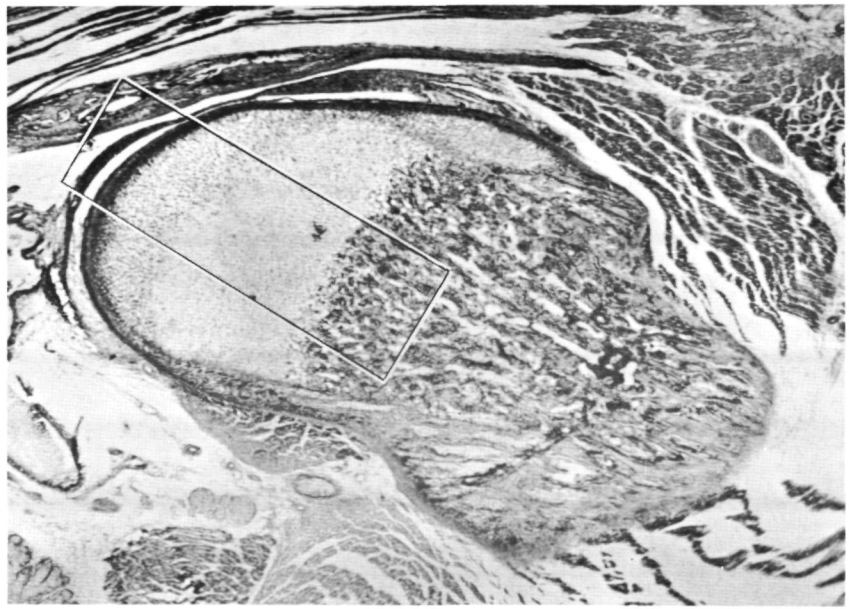
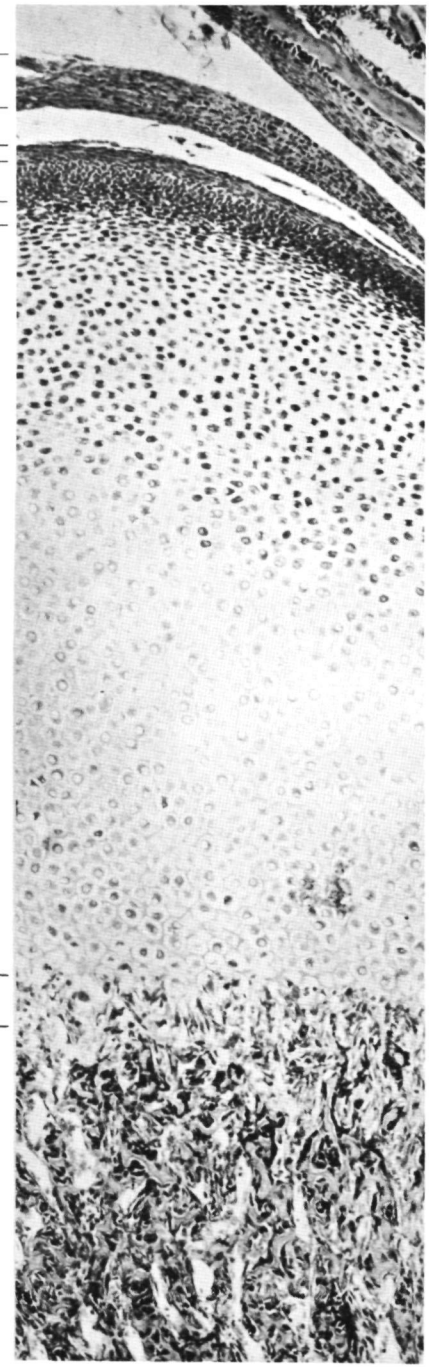


Figure 5.1
Parasagittal section through the mandibular joint of a 9-day rat
The right side of the figure is the anterior side. Inset: area reproduced at higher magnification in Fig.5.1a.
H.E.; Magnification: x 28.

the term chondrogenic cells. From the experiments with tritiated thymidine it was also clear that these cells differentiate into chondrocytes via the transitional layer. The fact that labelled cells do not migrate towards the articular layer may be interpreted as an expression of the dual function of the condylar process, viz. that of a growth centre and that of an articular process (Blackwood 1966).
The transitional layer is about as thick as the proliferating layer, but its cells are much larger and have a flat shape. Here the amount of intercellular matter is considerably greater. We might say that the proliferating layer, and perhaps the transitional layer as well, are chondrogenic.

Figure 5.1a ►
Parasagittal section through the mandibular joint of a 9-day rat
Enlargement of inset in Fig.5.1. The different layers and zones in the mandibular condyle are shown in detail.
H.E.; Magnification: x 115.


upper articular cavity
articular disk
lower articular cavity
1 articular layer
2 proliferating layer
3 transitional layer
4 zone of cartilage
5 zone of erosion
6 zone of cartilaginous trabeculae

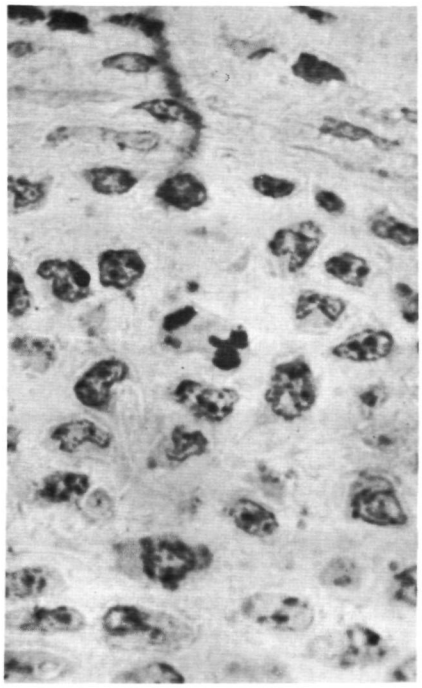


Figure 5.2
Detail of section through the mandibular condyle of an 11-day rat
The top of the figure shows part of the articular layer, at the bottom is the transitional layer, in the middle the proliferating layer. Note the differences in shape of the cells of these layers. In the centre of the Figure (in the proliferating layer) is a cell in anaphase. The cells in the proliferating layer divide with their spindle axes parallel to the articular layer. Preparation made by Mr. G.F.J.M. Vrensen, Electron Microscopical Laboratory (head: Dr. A.M. Stadhouders). The thickness of this section is 0.5 to 1 μ. Fixative: glutaraldehyde-OsO_4; embedding: Epon; staining: Tol.Blue in 2% Na_2CO_3, pH 12. Magnification: x 1050.

In the adjacent zone of cartilage, going from the transitional layer in the direction of the ramus, we observe a great change in the cell picture. Next to the transitional layer we get a section where the chondrocytes are of elliptical shape and much larger than the cells of the preceding layers. The dark stained nucleus seems polygonal. Characteristic for these cells are the vacuoles on the flattened side of the oval. The cells present a chaotic arrangement, and although their largest dimension is more or less parallel to the preceding layers, there are no columnar formations, such as occur in epiphyseal cartilage. These cells are largely concentrated in the posterior part of the condylar cartilage. More laterally, where the cartilage is enveloped in the perichondral tube, such cells are much less numerous.

There is doubt whether chondrocytes continue to divide in the cartilaginous zone. The answer to this problem is of major importance, linked as it is with the question whether the growth of condylar cartilage occurs solely as a result of deposition, that is, by addition from surrounding layers, or also by interstitial growth, caused both by frequent divisions of the chondrocytes and by the production of large mas-

ses of intercellular substance. In view of the fact that the young chondrocytes become more and more separated from each other by considerable amounts of this intercellular substance, it seems not unreasonable to suppose that they produce this substance themselves. But they remain isolated, not huddled in clusters or piled up in columns. Moreover in the experiments of Chapter 7, labelled chondrocytes were not observed 2 hrs after injection of tritiated thymidine. In epiphyseal cartilage on the other hand labelled chondrocytes were observed after 1 hr. (Kember 1960). These considerations seem to confirm Weinmann and Sicher's (1964) view that in the condylar process the cartilaginous growth is of a depositional character, and that no proofs of an interstitial nature have as yet been forthcoming.

Gradually the cells get larger and begin to show hypertrophy. Their nuclei increase in size and become round in shape. They only allow of scant granular staining. The amount of matrix is gradually reduced, but, inversely, becomes more and more capable of intense staining. Over a fairly wide layer, immediately adjacent to the erosion zone, the cartilaginous matrix has been calcified.

In the zone of erosion the picture is abundantly rich in cells. The cavities where the chondrocytes were situated, are broken open and around the remains of the matrix a large number of undifferentiated cells and also multi-nuclear cells and small blood vessels occur. The zone of erosion passes into the wide zone of the cartilaginous trabeculae, where bone is deposited by osteoblasts on to the calcified remnants of the cartilaginous matrix, the remains of which can be traced quite far into the ramus. Here, too, scattered multi-nuclear cells are found.

The last three zones greatly resemble the corresponding zones in the epiphyseal cartilage. Here, however, the picture is much more confused, the chondrocytes not being arranged in columns. We got the impression that, whereas the erosion zone of the epiphyseal cartilage displays intense vascularization, the condylar cartilage in this zone shows considerably less vascularization.

Divergent views are expressed about the nature of the processes taking place here and the origin of the various cells found in such an area. Maximow and Bloom (1953), McLean and Urist (1961), and also Holtrop (1964) suppose that not all the hypertrophying chondrocytes are lost, some survivors being capable of differentiating into osteoblasts and osteocytes. Trueta *et al.* (1960) contend that the cells from the blood vessel walls can differentiate into osteoblasts. Other authors think that undifferentiated connective-tissue cells, which invade such an area together with the blood vessels, are the predecessors of the osteoblasts

lining the remains of the cartilaginous matrix. For these cells various names have been proposed, such as spindle cells (Kember, 1960), reticular cells (Weinmann and Sicher, 1955) or osteoprogenitor cells (Young, 1962a). About the origin of the osteoclasts, too, different views are held (Tonna, 1960; Kember, 1960; Tonna and Cronkite, 1961; Maximow and Bloom, 1953; Weinmann and Sicher, 1955; Ham, 1965). They might develop from osteocytes, osteoblasts, undifferentiated connective-tissue cells, and even from chondrocytes.
Irving and Durkin (1965) assume a difference between epiphyseal cartilage and mandibular condylar cartilage as regards the erosion of hypertrophying chondrocytes. They postulate that in epiphyseal cartilage this erosion is to be traced back to the outpouchings of the small blood vessels adjacent to the hypertrophying chondrocytes, but that in the case of the mandibular condylar cartilage it is the osteoclasts which are responsible.

C. EXPERIMENTAL DESIGN

In the experiments described in the present chapter we used 3 groups of rats (1 experimental and 2 control groups).

Group 1, the experimental group, consisting of 24 4-day rats, originating from 3 litters. On all the animals a unilateral condylectomy was performed on the left side. The condyle was set free and immediately afterwards autologously implanted into the cerebrum. Operations were performed as described in Chapter 2 (2.C.3 and 2.C.4). One animal died a short time after the intervention.

Group 2, consisted of 22 4-day rats, on which condylectomy was also performed. However, instead of being implanted the freed condyles were immediately put in the fixative.

Group 3, comprised 3 litters of 11-day rats. The animals were killed and after fixation of the head the right-sided mandible was set free. In this way we obtained 21 mandibles, on which the required measurements could be carried out.

The experiment with group 1 was terminated 7 days after the operations. 23 Animals survived in good condition. Under ether anaesthesia, standardized radiographs of all the animals were made by means of a roentgen-cephalostat. The radiographs were taken in ventro-dorsal direction. Next, the animals were killed and the implants retrieved and fixed. In

order to set free the right and left mandible the rest of the head was likewise fixed. One implant was not retrieved; two right-sided mandibles, on being dissected out, were damaged to such an extent as to make them unsuitable for exact measurements.

When the operated animals were inspected, a clear mid-line deviation of the mandible towards the operated side was seen. Study of the ventro-dorsal radiographs confirmed this observation.

D. MEASUREMENTS

On the material obtained measurements were carried out as described in Chapter 2 (2.D.1). They are indicated in Figs. 5.3, 5.4, 5.5 and 5.6. The number of measurements performed would sometimes differ from the original number of animals in a group. This was due to the fact that a few specimens, on being set free, were slightly damaged.
In our drawings both of the controls of group 2 and of the implants the cut faces and the round articular sides were clearly seen.

In table 5A the measurements have been listed and their mean values and standard deviations given. In the legend to this table some of these figures are further elucidated.

Explanatory Note to Table 5A

Measurement No. 2:
The width of the cut face was measured at right angles to the longitudinal measurement No. 1 (Fig. 5.3);

Measurement No. 5:
The width of the cut face was measured at right angles to the longitudinal measurement No. 4 (Fig. 5.4);

Measurement Nrs. 3, 6:
The width at the articular side was measured at right angles to the longitudinal axis, at a distance of 6 mm* (on the drawing) from the point of intersection of the longitudinal axis with the outline on the articular side (Figs. 5.3 and 5.4).

Measurement Nrs. 7, 11, 15:
For all drawings of the mandibles of the experimental Group 1 and control Group 3 a tangent was constructed to the double concave outline

* In reality this means a distance of $\frac{1}{12}$ x 6 mm = 0.5 mm.

Table 5 A

Number (n), means (M) and standard deviations (SD) of measurements of mandibular condyle implants, control condyles, condylectomized mandibles and control mandibles * (cf. Figs.5.3 - 5.6)

Group	Measurement number	Specifications (see also Explanatory Note in text)	Measurement number	n	M	SD
implants from Experimental Group 1 (see Fig.5.3)	1	length of implant	1	21	26.34	3.05
	2	width of cut face	2	18	16.84	0.84
	3	width of articular side	3	18	15.73	1.33
condyles of 4-day rats from Control Group 2 (see Fig.5.4)	4	length of control condyle	4	22	16.87	1.93
	5	width of cut face	5	20	20.82	0.75
	6	width of articular side	6	22	18.35	0.81
right-sided mandibles of rats from Experimental Group 1 (see Fig.5.5)	7	from point B to condyle (longest distance)	7	20	129.36	6.70
	8	from tip of incisor to angular process (longest distance)	8	19	186.43	9.33
	9	width of articular side	9	20	20.34	2.52
	10	distance from alveolar process of molars to angular process	10	19	113.54	5.13
left-sided condylectomized mandibles of rats from Experimental Group 1 (see Fig.5.6)	11	from point B to amputation stump	11	21	94.37	5.07
	12	from tip of incisor to angular process (longest distance) (≙ 8)	12	19	185.36	8.26
	13	distance from alveolar process of molars to angular process (≙ 10)	13	19	112.15	5.21
right-sided mandibles of 11-day rats from Control Group 3 (the same as Fig.5.5)	14	from point B to condyle (≙ 7) (longest distance)	14	21	117.35	6.95
	15	from tip of incisor to angular process (longest distance) (≙ 8)	15	21	190.95	11.19
	16	width of articular side (≙ 9)	16	21	19.82	0.88
	17	distance from alveolar process of molars (≙ 10) to angular process	17	21	132.87	6.48

* The dimensions listed here (in millimetres) are those as measured on the tracings, and for this reason 12x the actual dimensions of the specimens.

of the lower border of the mandible. The projection to this tangent of the most posterior point of the concave outline of the alveolar process on the anterior side of the first molar determined the point B. From B we measured on the right-side mandible the longest distance towards the articular side of the condyle (measurements Nrs. 7, 14). The angle A between the tangent and the line B/condyle (No. 7) was copied from the rightsided mandibles of Group 1 on to the drawing of the left-sided condylectomized one. Thus measurement No. 11 is the distance between B and the point of intersection with the outline of the amputation stump.

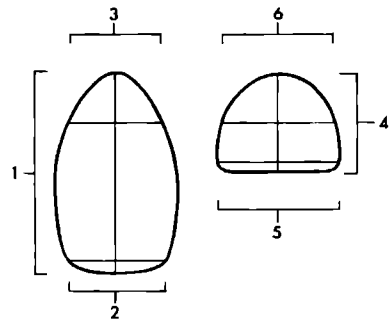


Figure 5.3 Figure 5.4

Figure 5.3
Tracing of an implant of the mandibular condyle, 7 days after autologous intracerebral implantation on the 4th day (implant from experimental group 1)

At the top the articular side; at the bottom the cut face. 3 Dimensions were determined on each tracing: No.1: length of the implant; No.2: width of the cut face; No.3: width of the articular side, measured at 6 mm from the top of the outline. This figure may be compared with Fig.5.4. See also Table 5A and its Explanatory Note.

Figure 5.4
Tracing of a mandibular condyle of a 4-day rat from control group 2

At the top the articular side; at the bottom the cut face. Three dimensions, comparable to those of the condylar implants after 7 days of implantation, were determined on each tracing: No.4: length of the control condyle; No.5: width of the cut face; No.6: width of the articular side, measured at 6 mm from the top of the outline.

This figure may be compared with Fig.5.3. See also Table 5A and its Explanatory Note.

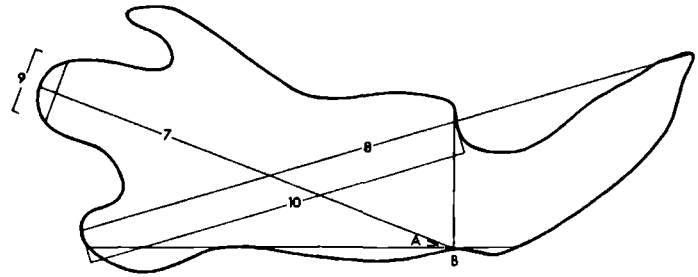


Figure 5.5

Tracing of a right-sided mandible of an 11-day rat from experimental group 1

Four dimensions were determined No.7 from point B to condyle (No.14), No.8. from tip of incisor to angular process (No.15), No.9. width of articular side, measured at 6 mm from the outline (No.16), No.10· distance from alveolar process of molars to angular process (No.17).

Corresponding dimensions (numbers between brackets) were determined on the tracings of the right-sided mandibles of non-operated 11-day rats from control group 3.

This figure may be compared with Fig.5.6. See also Table 5A and its Explanatory Note.

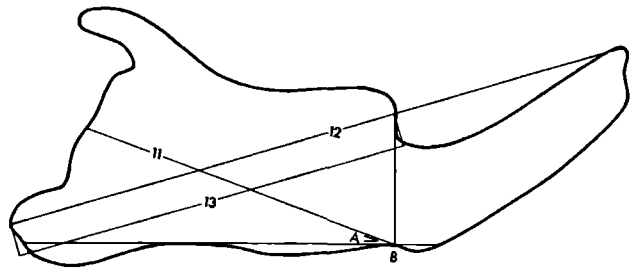


Figure 5.6

Tracing of a left-sided operated mandible of an 11-day rat from experimental group 1, 7 days after unilateral condylectomy on the 4th day

Three dimensions were determined on the tracings· No.11: from point B to amputation stump, No.12 from tip of incisor to angular process, No.13 distance from alveolar process of molars to angular process.

The angle A was copied from the right-sided mandible to the left-sided condylectomized one.

This figure may be compared with Fig.5.5. See also Table 5A and its Explanatory Note.

Measurement Nrs. 9, 16:

The width of the articular side was measured perpendicularly to the line B/condyle (measurements Nrs. 7 and 14 respectively) at 6 mm

Table 5 B

Significance of the differences between various measurements * carried out on condylar implants, left and right mandibles of Group 1, control condyles of Group 2, and right mandibles of Group 3

Differences tested by Student's t test or Welch's test for two samples	
measurements compared	differences
4- 1 **	+++
5- 2	+++
6- 3	+++
9- 6	+++
7-14	N.S.
8-15	N.S.
9-16	N.S.
10-17	N.S.
17-13	+

Tested by Student's test for pair differences			
measurements compared	mean value	SD	differences
9- 3	4.61	2.63	+++
7-(1+11)***	8.71	3.47	+++
8-12	1.07	2.51	N.S.
10-13	1.39	1.85	+++

\+ at the 5% level ($0.01 < p \leqslant 0.05$)
++ at the 1% level ($0.001 < p \leqslant 0.01$)
+++ at the 1‰ level ($p \leqslant 0.001$)
N.S. not significant

For meaning of numbers see Table 5A and its explanation (see p.59-60).

* The dimensions listed here (in millimetres) are those as measured on the tracings, and for this reason 12x the actual dimensions of the specimens.

** The measurement first placed is the larger of the two.

*** (1 + 11) is the sum of the measurements No.1 and No.11.

distance‡ (on the drawing) from the outline (Fig. 5.5).

Measurement Nrs. 10, 13, 17:

On the lines drawn for the measurements Nrs. 8, 12, 15 the longest

‡ In reality this means a distance of $\frac{1}{12}$ x 6 mm = 0.5 mm.

distance was measured between the intersection of these lines with the anterior side of the alveolar process of the first molar and their intersection with the outline of the angular process.

* * *

Table 5B lists the significance of the differences between the measurements compared.

As is clear from Tables 5A, 5B, compared with the controls of Group 2 the implants after the 7-day implantation period showed considerable increase in length. The mean length of the controls was 16.87, of the implants 26.34. All the implants are longer than the longest control. On the other hand, width dimensions were reduced. The width of the articular side of the implants was not only smaller than the same dimension in the non-operated 11-day right-sided mandibles, but also smaller than the width of the articular side of the 4-day controls of Group 2. The same phenomenon occurred on the side of the cut face. Here, too, the implants were appreciably smaller than the controls (Figs. 5.3, 5.4).

The length of the right-sided mandible (7) of the operated animals, measured from B, was significantly greater than the sum of the length of the implant (1) and the corresponding length of the condylectomized left mandible (11; Table 5B). Yet the sum of the length of the implant and the measured length of the condylectomized mandible (1+11) constituted as much as 91.6% - 94.9% of the corresponding length of the right-sided mandible (7).
When the other dimensions of the left and right mandibles of Group 1 were compared, the longest distance between the point of the incisor and the angular process (8-12) was not significantly different. We found a significant difference, however, for the distance between the anterior outline of the alveolar process of the molars, and the angular process (10-13). This dimension was observed to be slightly smaller in the operated mandibles. To the fact that we did find a significant difference for one dimension and not for the other, is to be attached little importance on account of the distribution of the figures (Compare Table 5B).

Finally, on a basis of 4 different measurements the intact right-sided mandibles of the operated rats from Group 1 were compared with the corresponding mandibles of rats of control Group 3. No differences were found.

E. HISTOLOGICAL INVESTIGATION

Microscopical serial sections were made of all the implants from Group 1, and of all the control condyles from Group 2. The direction in which the sections were cut was parallel to the plane in which the measurements were carried out, in order to obtain sections of maximal length and width.

Table 5C
Presence of trabeculae at cut faces of mandibular condyle implants and of control condyles

	number	assessed	trabeculae
implants (Group 1)	21	19	4
controls (Group 2)	22	19	5

Histological investigation of the controls showed that we had been fairly successful in determining the sites for condylectomy (Table 5C). Out of the 19 controls that could be assessed, 5 turned out to contain some cartilaginous trabeculae, which, however, in all these 5 specimens only occurred in part of the cut face (Fig. 5.7). Of the implants, 4 specimens displayed a number of trabeculae at the cut face. A deviation in the sectional plane, developed in the course of histological processing, prevented accurate assessment of this aspect in 3 controls and 1 implant. 1 Implant was lost in processing.
In the controls, around the hypertrophying cartilage adjacent to the cut face, there was a small strip of membranous bone, its periosteum rich in cells. Towards the articular surface this gradually changed into a perichondrium. In all specimens the disk had disappeared. Occasional shreds of muscle tissue were attached to the periosteum or the perichondrium. In the periosteum small bloodvessels were seen.

In all the implants the cut face and the articular side were clearly distinguishable, not only from the external shape of the sections, but above all by the highly different cellular picture for the two sides. Enveloping the implant, although often torn, a connective-tissue capsule could be observed, having a thickness of 3-4 cells (Fig. 5.8).

On the articular side of all implants a changing mass of cartilage had developed. All the zones that are distinguished in a normal condyle *in situ* were present: articular layer, proliferating layer, transitional layer, and the cartilaginous layer; these layers, however, had become considerably narrower. It is normal for the zones to be reduced with advancing age, but not to any extent like this (Figs. 5.9 and 5.10). The characteristic elliptical young cartilaginous cells, presenting a polygonal nucleus and the lateral vacuoles surrounded by a large mass of intercellular matter, were in the implants almost entirely absent. On the other hand, such cells abundantly occurred in the caudal part of the 4-day control material. As in normal cases, the proliferating layer - abundant in cells - showed a regular decrease in thickness. It had its maximum thickness at the highest point of the condyles (Fig. 5.8).

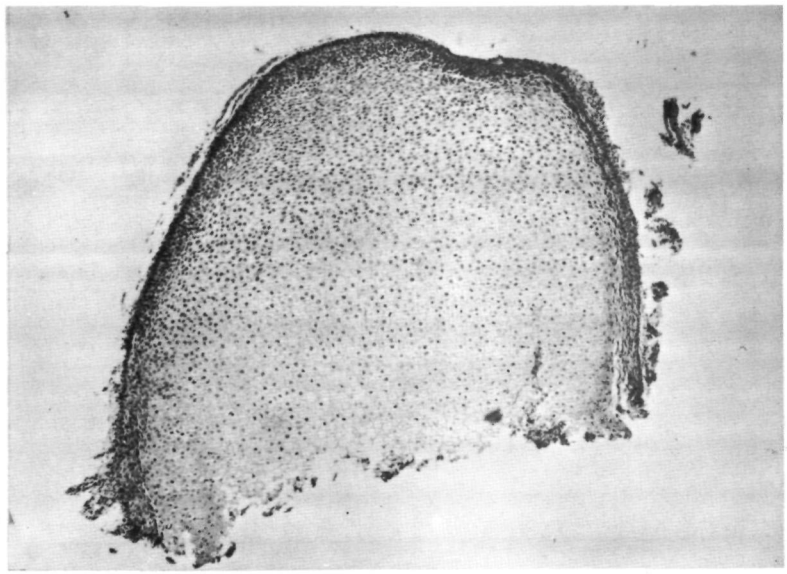


Figure 5.7
Parasagittal section through a mandibular condyle of a 4-day rat from control group 2
At the top the articular side; at the bottom the cut face. This specimen does not show any cartilaginous trabeculae on the cut face (see also Table 5C).
H.E.; Magnification: x 44 (same enlargement as Fig.5.8).

In numerous implants blood vessels had developed, which vessels, surrounded by a large mass of connective-tissue cells, penetrated as strands into the cartilage on the articular side (Fig. 5.11).

In all implants endochondral ossification had developed. Frequently the picture would be highly irregular, but comparable to that of a similar area in a condyle *in situ*. The remnants of the matrix were enveloped by bone and osteoblasts. Intense vascularization had set in. Blood vessels appeared to be distended here and surrounded by large numbers of mesenchyme cells. Bone marrow was absent. A great many multi-

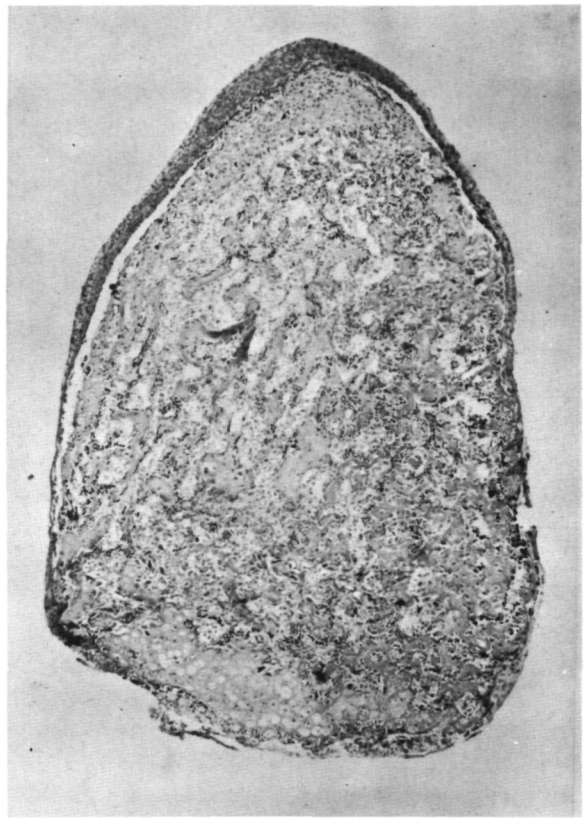
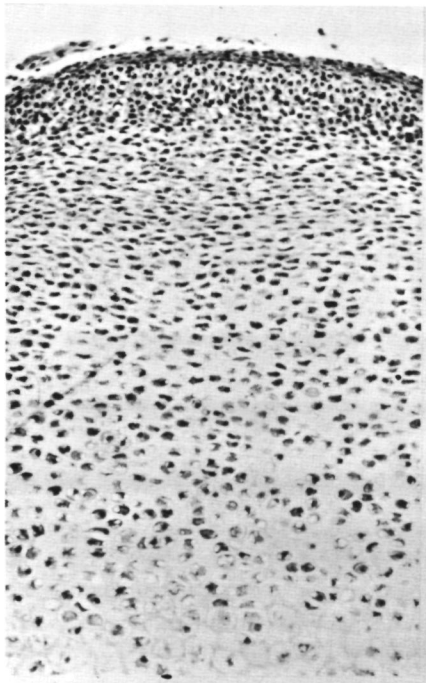


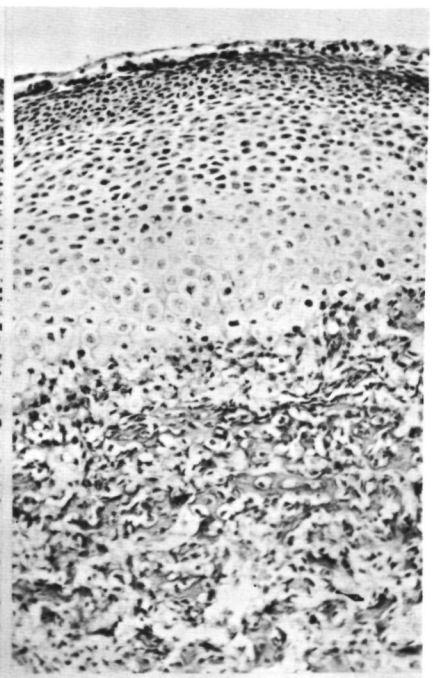
Figure 5.8
Parasagittal section through an implant of the mandibular condyle of an 11-day rat from experimental group 1, 7 days after autologous intracerebral implantation on the 4th day

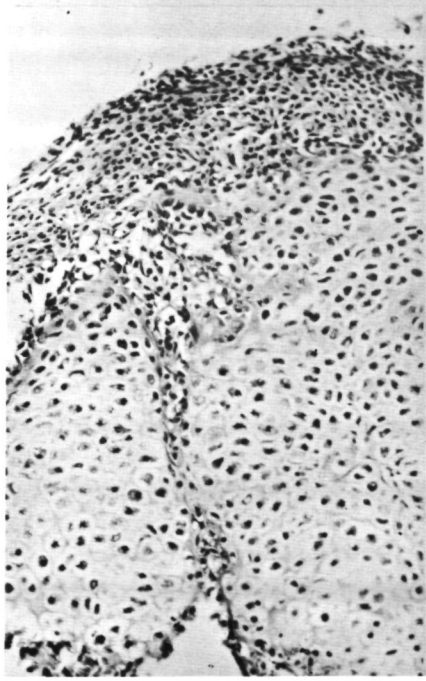
At the top the articular side; at the bottom the cut face. All the zones distinguishable in a normal condyle *in situ* are present. Here, however, these zones have become considerally narrower. Endochondral ossification has developed in the centre of the implant. On the side of the cut face a layer of intensely hypertrophic cartilage is visible. This figure may be compared with Figs.3.4; 4.7; 5.7
H.E. Magnification: x 44 (same enlargement as Fig.5.7).

5.9

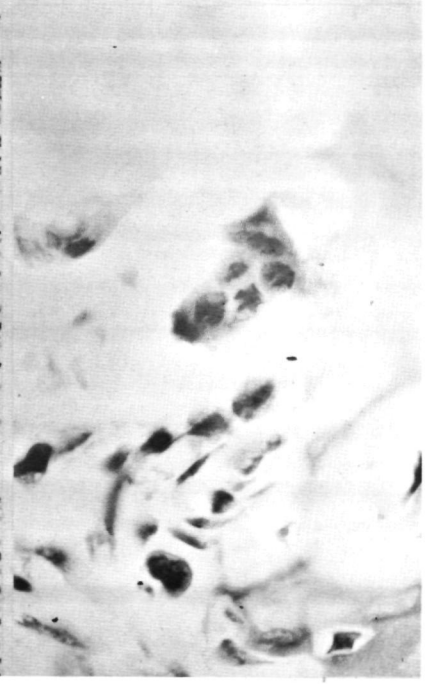


5.10





5.11



5.12

nuclear chondroclasts were observed along the erosion zone of the articular cartilage (Fig. 5.12) and, besides, scattered throughout the area of endochondral ossification. The outside walls of the implants as a rule showed a very narrow bony layer and periosteum, which often were penetrated by blood vessels (fig. 5.8). Also on account of the manifest tendency for the cartilage on the articular side to disappear, we gained the impression that with a longer implantation period this bony layer would have enclosed all of the cartilage.

On the side of the cut face was a layer of intensely hypertrophying cartilage (cf. Fig. 5.8), varying in thickness from implant to implant. In some implants it was about 10-12 cells thick; in others only 4 or 5, the latter layer often being penetrated by blood vessels. At the interior demarcation line of this layer likewise numerous chondroclasts were observed.

◄ *Figure 5.9*

Detail of a parasagittal section through a mandibular condyle of a 4-day rat from control group 2

This figure may be compared with Fig.5.10.
H.E.; Magnification: x 104 (same enlargement as Figs.5.10; 5.11).

Figure 5.10

Detail of a parasagittal section through an implant of the mandibular condyle of an 11-day rat, 7 days after autologous intracerebral implantation on the 4th day (experimental group 1)

All the zones distinguishable in a normal condyle *in situ* are present. Here, however, these zones have become considerably narrower. It is normal for the zones to be reduced with advancing age, but not to any extent like this. Highly irregular endochondral ossification is shown in the lower half of the figure. This figure may be compared with Fig.5.9.
H.E.; Magnification: x 104 (same enlargement as Figs.5.9; 5.11).

Figure 5.11

Detail of a parasagittal section through an implant of the mandibular condyle, 7 days after autologous intracerebral implantation on the 4th day (experimental group 1)

In numerous implants blood vessels had developed, which vessels, surrounded by a large mass of cells, penetrated as strands into the cartilage on the articular side.
H.E.; Magnification: x 104 (same enlargement as Figs.5.9; 5.10).

Figure 5.12

Detail of a parasagittal section through an implant of the mandibular condyle, 7 days after autologous intracerebral implantation on the 4th day (experimental group 1)

A great many multi-nuclear chondroclasts are observed along the erosion zone of the articular cartilage.
H.E. Magnification: x 960.

Some of the cells in this zone of intensely hypertrophic cartilage appeared to lie loose in their matrix cavity and displayed with H/E a dark-stained nucleus. In other matrix cavities more than one nucleus could be seen, sometimes 2, sometimes 4. With Toluidine Blue there was hardly any staining of the matrix of this zone. It is likely that the 4 implants showing trabeculae on the side of the cut face, already contained them when the experiment began (cf. Table 5C). The trabeculae were lined with osteoblasts, but otherwise these implants presented the same picture as the others.

In 2 implants a much more extensive bone formation was found on the outside. Both had been retrieved near the cranial vault, but they had not become attached to it. A similar phenomenon was observed in one costal cartilage implant (Chapter 3.C). In Chapter 10 we shall discuss a possible reason for this.

F. DISCUSSION AND CONCLUSIONS

Statistical analysis of the findings from the measurements confirmed our observations that over the implantation period of 7 days the implants had considerably increased in length. The width dimensions, however, had been appreciably reduced. Not only were they smaller than the corresponding dimensions of the condyles of non-operated 11-day rats, but they were even smaller than those of 4-day controls. Evidently, a condylar formation comparable to that which occurs under normal circumstances, was not realized.

The sum of the length of the implant and of the amputated left-sided mandible, measured in the direction of condylar growth, amounted to 91.6% - 94.9 % of the length of the non-operated right-sided mandible. Such a high percentage is unlikely to be due merely to the longitudinal growth of the implant. On superimposing the tracings no difference was found between the outlines of left and right mandibles as regards the points required to determine point B. Hence it is likely that reparative processes and perhaps also the formation of a nearthrosis on the amputated ramus should have contributed to this high percentage. This hypothesis is supported by the findings of other workers. Jolly (1961) after condylectomy in adult rats found regeneration attended by formation of cartilage at the end of the amputated ramus. Jarabak (1953) after condylectomy in 30-day rats likewise observed chondrogenesis. He

compared this process to the formation of cartilaginous callus after fracture of a long bone. Gianelly and Moorrees (1965), however, in a similar experiment on rats found only granular tissue at the end of the amputated ramus. With unilateral condylectomy in pigs Kantack (1965) was able to ascertain the formation of a new condyle. Kusen (1960) saw in monkeys the formation of cartilage at the end of the amputated ramus. In unilateral condylectomy experiments on dogs Peskin and Laskin (1965) were able to observe the formation of a new condyle covered with a layer of fibrous connective tissue, but there was no formation of cartilage.
We do not know of any experiments in the literature in which 4-day rats were used. Moreover, in the above-mentioned investigations on rats it was always a bilateral condylectomy that was performed. This fact, together with the considerable difference in age, makes a comparison difficult. Nevertheless, we feel justified in assuming that on the amputated side such processes have occurred as to influence the longitudinal dimension referred to (11) in a positive sense. Unfortunately, this aspect was not further looked into histologically.

There was a slight delay in longitudinal increase of the angular process on the operated side. Apart from deviations due to the absence of the condylar process, measurements did not indicate the presence of any abnormalities in other parts of the left-sided mandible. It would seem, then, that mandibular development between 4 and 11 days takes place fairly independently of the condylar process. All workers performing condylectomy in rats of more advanced age found unmistakable anomalies in the shape of the mandible. Such anomalies consisted in a deviating growth of the angular process and a marked excrescence of the coronoid process. Das et al. (1963) investigated by means of vital staining and radiography the deposition pattern in the mandibles of condylectomized rats of 35 days. The authors interpreted the changes in shape as an adaption to muscular hypertrophy, as a result of which the bony attachments were enlarged.

Of any influence of the operation, revealing itself in delay in growth, as could be demonstrated in the metacarpal-experiment, no traces were found. No difference was discernible between the dimensions of the right mandibles of operated and non-operated control rats. Must then the metacarpal-experiment to be considered a more severe intervention? Might the nutritional uptake of the animals have been inhibited by the restrictions of their movements? Our findings seemed also to suggest

that in the condylectomized rats the act of suction was but little impaired. It is remarkable that with the methods used in this study no effect of the unilateral condylectomy on the non-operated side could be demonstrated.

On the basis of the histological investigation we were able to form a notion of the processes which had taken place in the condylar implants. On the cut face there persisted a layer of hypertrophic chondrocytes. The absence of metachromasia of the matrix in this layer after implantation suggests the occurrence of changes in its composition. Similar zones of hypertrophic cartilage were found in costal cartilage implants (Chapter 3, Fig. 3.5).
This kind of achromatic cartilage was also seen in metacarpal implants. Illustrations provided by Felts (1961) show, as has been mentioned before, a similar phenomenon. Hence it is clear that at the cut face the hypertrophic cartilage behaves in a similar way as in implants of a totally different origin. However, there is no absolute certainty that this zone in the condylar implants did indeed consist of the cartilage originally situated at the cut face. The next chapter will deal with this in more detail. Why there was no further break-down of these chondrocytes we were unable to find out, but presumably this is largely due to the avascular period immediately following upon implantation, as already referred to in Chapter 3.
The endochondral ossification found in the centre of the implants, was irregular. It should be considered, though, that even sections of normal condyles will not present the orderly picture that is so characteristic of most other sites of endochondral ossification. The very strong vascularization of this part of the implants was remarkable. It was so intense as to give the impression that it was in excess of the normal condition. Vascularization affects the course of endochondral ossification considerably. Trueta and Amato (1960) found a widening of the hypertrophying zone in epiphyseal disks after metaphyseal ischaemia. Metaphyseal hyperaemia (Hansson and Wiberg, 1963) did not induce a reduction of the hypertrophying zone, but caused narrowing of the zone of cell columns. Hansson and Wiberg concluded that there were fewer cells per column and that since the hypertrophying zone had not become narrower, hypertrophy in the chondrocytes had a more rapid course.

The histological structure on the articular side resembled that of a condyle *in situ*, although all zones were very much narrower. We supposed that longitudinal growth had taken place on this side, but it is not

certain whether this had happened in the same way as *in situ*. The possibility must not be ruled out that owing to the fact that the articular surface had been exposed to quite abnormal circumstances, no more cell divisions in the proliferating layer should have occurred. Another possibility is a disturbance of cell differentation, originating from the proliferating layer. An indication of this might be found in the presence of the strands of connective tissue cells, together with blood vessels, which strands penetrated the cartilage. In the articular cartilage of implanted metacarpals this phenomenon was nowhere observed.

Longitudinal growth might be the result of the production of intercellular matter by a limited number of young chondrocytes which had retained this capacity. Experiments described in the next chapter will shed some light here. In this connection an interesting experiment by Holtrop (1964) on mice deserves mentioning. Costal cartilage fragments containing the small cell zone as well as the columnar one, stopped growing in length after circa 2 weeks. If after an implantation period of 3 weeks such implants were set free, the ossified part cut off, and the cartilaginous part re-implanted, longitudinal growth and endochondral ossification would start again. From this, Holtrop concluded that the presence of the ossified part was an inhibitory factor.

From the experiments as described here it was evident anyhow that the balance between the production and the break-down of chondrocytes had been seriously upset. Presumably production lagged or had stopped altogether, while the break-down had increased, resulting in a double disadvantage for chondrogenesis.

Summarizing, we may state that the mandibular condyle implants, over the 7 days that they were autologously implanted intracerebrally, had grown in length. Besides, the process of endochondral ossification had been initiated and the mass of cartilage appreciably reduced.
Nevertheless, it was not clear from these experiments whether the cartilage occurring at the cut face of the implants had already been there when the experiments began. Nor had it become clear whether the longitudinal growth in the implants was comparable to the one in a normal, non-implanted condyle.
For further investigation into these two problems a number of experiments were performed, which will be described in Chapters 6 and 7.

Chapter 6

AUTOLOGOUS IMPLANTATION OF THE MANDIBULAR CONDYLE, COMBINED WITH TETRACYCLINE VITAL STAINING

A. INTRODUCTORY

It was supposed in the previous chapter that the hypertrophic cartilage found in the 7-day condylar implants on the side of the cut face was, in fact, cartilage already existing at the moment of implantation; in other words, that this hypertrophic cartilage observed in the implant was old cartilage.
We can prove the correctness of this hypothesis by marking the cartilage prior to implantation. In the condyle of the 4-day rat a progressive calcification of the matrix may be observed over a wide zone of the hypertrophic cartilage. Such calcifying cartilage can be indicated by a bone marking agent. For this purpose we used tetracycline, which was injected a short time before the operation. When the implant is introduced, it contains marked cartilage near the cut face. If after the test period of 7 days the tetracycline is retraced in the matrix of the hypertrophic cartilage on the side of the cut face of the implants, the hypothesis referred to above may be considered to have been proven. At the same time it might be concluded that the increase in longitudinal growth is due to processes occurring on the articular side of the implant. In the next chapter, experiments will be reported which aim specifically at the latter aspect.

Tetracycline is one of those bone marking agents - alizarine is another of them - which have a special affinity for calcifying tissues. According to Steendijk (1964) the fixation of tetracycline in the bone and in calcifying cartilage takes place through a process of surface adsorption. In animals as well as in man high and long-term doses may result in disturbances of the calcification mechanism (Bevelander, 1964; Sognnaes *et al.*, 1964). In a great many children discolourations and malformations of deciduous and permanent teeth have been observed, which are alleged to have been caused by tetracycline. As a rule such abnormalities are not found until years after administration of the drug, when

those teeth are breaking through that had been forming at the time of administration. The possibility that tetracycline might be a growth stimulant was investigated by Grewe and Felts (1962) on isologously implanted humeri of neonatal mice. The specimens treated with tetracycline prior to implantation proved to be of greater length, which fact was attributed to an enhanced healing of the implant into the host's vascular bed. An antibiotic effect could not be ruled out, however.

Tetracycline, giving off a yellow fluorescence, is easily located under the fluorescence microscope.

B. EXPERIMENTAL DESIGN

When setting up this experiment we started from the supposition that calcifying tissues a few hours after administration of tetracycline will no longer be marked by this substance. To be on the safe side, the implants were not set free until 24 hrs after injection.
7 Rats (litter-mates) were given a tetracycline injection on the 3rd day after birth (Chapter 2 E.1). 24 Hrs after administration in 4 rats the mandibular condyle was autologously implanted into the cerebrum. The 3 other animals were used as controls. On these rats condylectomy was performed and the condyles immediately fixed. The experiment lasted 7 days.

C. HISTOLOGICAL EXAMINATION

The rats survived the experiment in good condition. Macroscopically, all the 4 retrieved condylar implants presented the picture as described in Chapter 5. The same was also seen under the microscope. Under the fluorescence microscope traces of the tetracycline were located in the calcified matrix of the hypertrophic cartilage, on the side of the cut face. (Fig. 6.1). Tetracycline fluorescence was solely found in this part of the implants. In view of the slightly irregular picture it is likely that abnormal processes have occurred in the matrix of this cartilage; about the nature of such processes we can only guess. A few of the sections were stained according to von Kossa, to demonstrate the calcified areas. Thus the calcified matrix was easily discerned.
As could be expected, in the sections of 4-day control condyles tetracycline was found on the side of the cut face.

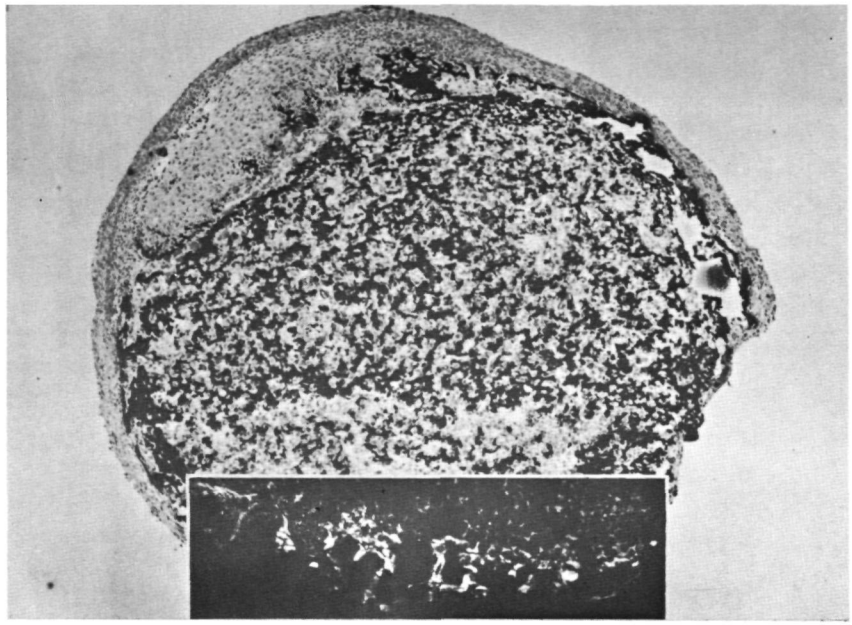


Figure 6.1
Section through an implant of the mandibular condyle,
7 days after autologous intracerebral implantation on the 4th day
To mark the calcifying matrix of the hypertrophic cartilage on the side of the cut face, tetracycline was administered 24 hrs prior to the operation on the 4th day. Note the extensive calcification of the implant as shown by von Kossa's staining. Tetracycline fluorescence was only found on the side of the cut face (inset).
Inset: part of the cut face showing the tetracycline fluorescence. See also Fig.5.8.
Undecalcified section; von Kossa; Magnification: x 46.

D. DISCUSSION AND CONCLUSIONS

In the experiment described in this chapter, a tetracycline injection was given, preceding autologous intracerebral implantation of the mandibular condyle. This was the way in which we marked the calcifying matrix of the hypertrophic cartilage prior to implantation. Our problem here was whether the zone of hypertrophic cartilage, found on the side of the cut face after a 7-day implantation period, had already existed at the beginning of the experiment.
Macroscopical and microscopical findings were in agreement with those

as described in Chapter 5.
The presence of the tetracycline in the zone of hypertrophying cartilage on the side of the cut face was clearly discernable both in the 4-day controls and in the implants that had been retrieved after 7 days. Hence we may infer that this cartilage corresponds to the cartilage which at the moment of implantation is found at the cut face. It is highly improbable, then, that the great increase in length of the condylar implants should be due to activities on the side of the cut face. From the experiment it may concluded that the processes responsible for the increase in length should be sought on the other side. In the next chapter we shall describe how by means of tritiated thymidine experiments we tried to find out in what way this longitudinal growth actually takes place.

Chapter 7

AUTOLOGOUS MANDIBULAR CONDYLE IMPLANTATION, COMBINED WITH TRITIATED THYMIDINE AUTORADIOGRAPHY

A. INTRODUCTORY

In the preceding chapter it was concluded that in the condylar implants longitudinal growth on the side of the cut face is highly improbable. The question remains, however, in how far the processes on the articular side, which may be held responsible for the increase in length of the implants, are comparable to the processes occurring in a normally growing mandibular condyle. For a detailed study of this point an experiment with tritiated thymidine was undertaken*.

Thymidine is one of the components in the formation of DNA. When thymidine labelled with the isotope tritium is injected, it is taken up by the nuclei of cells preparing for mitosis. In mitosis a duplication of DNA takes place, in the course of which the injected thymidine is built in. Histological sections of injected animals are brought into close contact with a photographic emulsion. During the period of development dark silver grains are formed in the emulsion owing to ionisation by the short tracks of beta particles emitted by the tritium. This provides microscopic localisation of the radio-active material.
Since the introduction in the 50's of this new cyto-histological technique a spate of literature has appeared about its many aspects and possibilities. Here are a few general remarks, relevant to our purpose.
It is assumed that after some two hours ^{3}H-TdR is either incorporated in the body or eliminated from it. Once cells are labelled with ^{3}H-TdR, they cannot get rid of it. In cell division the radio-active material is distributed over both daughter-cells and thus 'diluted'. When cells die, the material can be taken up again by other cells. This method can be used for qualitative as well as for quantitative purposes. Qualitatively, it is by tracing the fate of zones of labelled cells or by identifying the precursors of certain cell types. Quantitative assessment is based on

* Hereafter abbreviated as ^{3}H-TdR

counting the number of labelled cells or the number of grains per cell. (Young, 1962a, b; Kember, 1960; Blackwood, 1966; Tonna, 1960; Tonna & Cronkite, 1961; Folke & Stallard, 1966).
Injecting ^{3}H-TdR during our experimental period enabled us to determine whether in the proliferating zone of the implants cell division persists. Furthermore, by drawing out the period between the injection and the death of the animal, we can also discover whether the new-formed cells are still differentiating into chondrocytes and, if so, what happens next to them.

B. EXPERIMENTAL DESIGN

For this experiment a litter of 8 rats was used. When they were 4 days old, on all rats an autologous intracerebral implantation of the mandibular condyle was performed, as described in Chapter 2 (2.C.3 and 2.C.4). 3 Rats died prematurely.
In order to get a good insight into what was occurring during the latter half of the 7 day-implantation period, the experiments were staggered over the last 3 days. The 5 animals which survived in good condition, were dealt with as follows:

1. One rat was injected 4 days after the operation and killed 2 hrs afterwards.
2. Two rats were injected 5 days after the operation and killed 2 x 24 hrs afterwards.
3. Two rats were injected 6 days after the operation and killed 24 hrs afterwards.

After terminating the experiment the implants were retrieved in the usual way. The right-sided mandibular condyles were likewise set free and served as controls. The material was processed as described in Chapter 2 (2.E.2).

C. HISTOLOGICAL INVESTIGATION

1. In the right-sided control condyle of the rat that had been killed 2 hrs after injection, over a fairly well-defined area of the proliferating zone a large number of clearly labelled cells could be observed. The cells of the transitional and cartilage layers were unlabelled. In the area of endochondral ossification a large number of labelled mesenchyme cells were found. Osteoblasts, osteoclasts, and osteocytes were

unlabelled. It was remarkable that in the articular layer there was only a scatter of labelled cells.

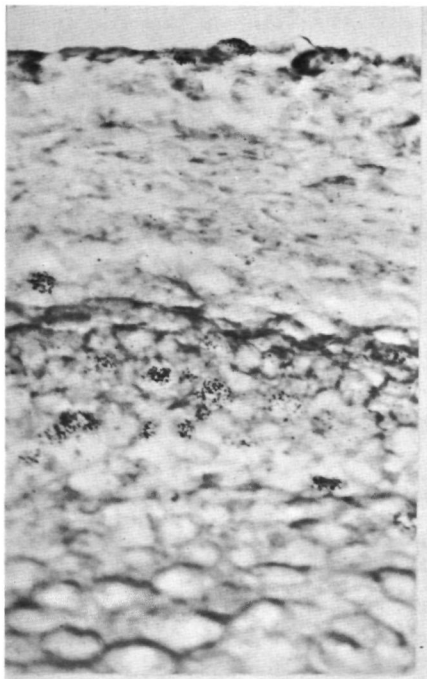
In the implant the endochondral ossification together with the invading vascularization had not yet begun at 4 days after the operation. Here, too, on the articular side a proliferating layer could be seen, with a large number of heavily labelled cells (Fig. 7.1). In the articular layer, as in the right-sided control condyle, only very few cells were labelled. Remarkably, also in the old hypertrophic cartilage on the side of the cut face some clearly labelled cells were seen (Fig. 7.2).

2. The right-sided control condyles of the two rats that had been given ^{3}H-TdR 5 days after the operation and which had been killed after 2 x 24 hrs, presented the following picture:
The labelled cells were scattered through the proliferating and transitional layers, and through the cartilage up to the initial hypertrophying zone. Here, too, only occasional labelled cells were found in the articular layer.
Morphologically, the implants of these two rats presented the picture described above. On the articular side, labelled cells were found in the proliferating and transitional layers and likewise throughout the area of the hypertrophying cartilage. We even perceived a few cells whose walls had already been eroded, while the grains were still clustered together (Fig. 7.3).
Numerous labelled osteoblasts and osteocytes were seen; osteoclasts and chondroclasts having one or more labelled nuclei were also observed. Although the granules were not counted, we got the impression that the implant cells were heavier labelled than the control condylar ones.

3. Six days after the operation and 24 hrs after the ^{3}H-TdR injection the labelled cells in the controls were scattered through the proliferating and transitional layers and the initial cartilage.

Morphologically, the implants presented the familiar picture. On the articular side labelled cells were present in the proliferating layer and in the transitional layer up to the initial hypertrophying zone, some 7-8 cells removed from the erosion zone of the hypertrophying cells. In the area of endochondral ossification numerous mesenchyme cells and osteoblasts were labelled. No labelled osteoclasts or chondroclasts were found (Fig. 7.4).

7.1



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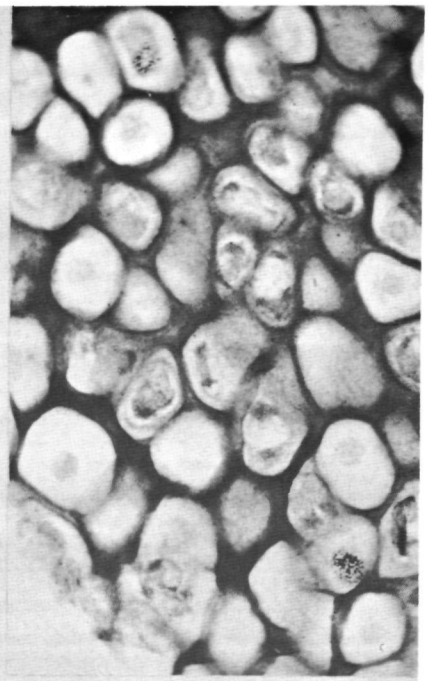

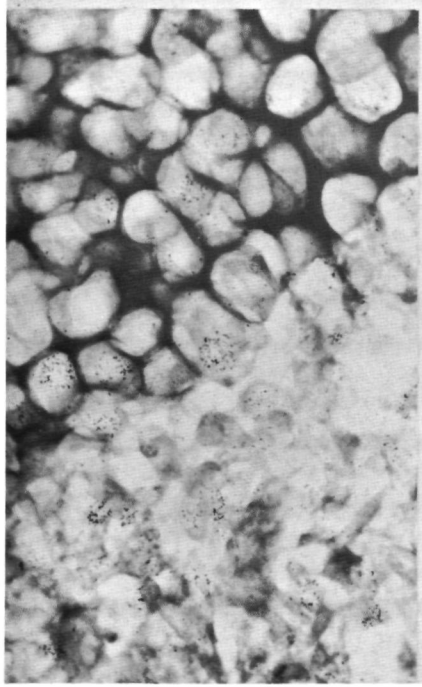

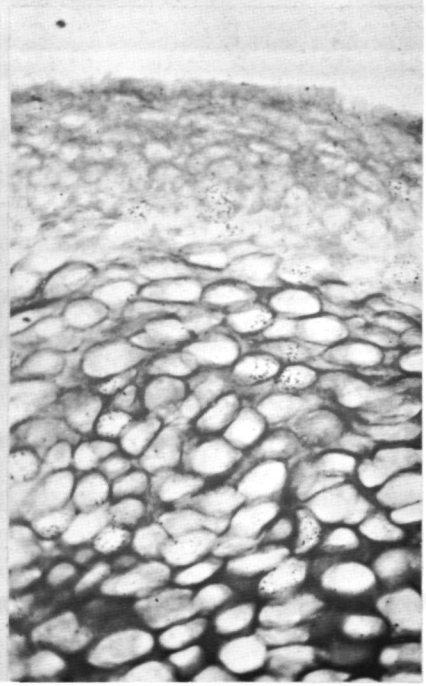

7.3

7.4

Figure 7.1

◄ *Section through an autologously, intracerebrally implanted mandibular condyle*
Implantation on the 4th day. The rat was injected with tritiated thymidine 4 days after the operation and killed 2 hrs afterwards. This autoradiogram shows detail of the articular side of the implant. In the proliferating layer a large number of heavily labelled cells are visible. In the articular layer only very few cells are labelled.
Tol. Blue, Magnification. x 333.

Figure 7.2
Detail of the old hypertrophic cartilage on the side of the cut face of the same implant as partly shown in Fig.7.1
Some clearly labelled cells are seen in this area. See also Fig.5.8.
Tol. Blue, Magnification x 333.

Figure 7.3
Section through an implant of the mandibular condyle
Autologous intracerebral implantation on the 4th day. The rat was injected with tritiated thymidine 5 days after the operation and killed 2 x 24 hrs afterwards. This autoradiogram shows part of the zone of hypertrophic cells and zone of erosion of the articular cartilage of the implant. Labelled chondrocytes are visible in the zone of erosion. In a few cells, whose walls have already been eroded, the grains are still clustered together.
Tol. Blue. Magnification x 333.

Figure 7.4
Section through an implant of the mandibular condyle
Autologous intracerebral implantation on the 4th day. The rat was injected with tritiated thymidine 6 days after the operation and killed 24 hrs afterwards. This autoradiogram shows part of the articular side of the implant. Labelled cells are present in the proliferating layer and in the transitional layer up to the initial hypertrophying zone.
Tol. Blue; Magnification x 333.

D. DISCUSSION AND CONCLUSIONS

Two hours after the ^{3}H-TdR injection on the 4th day of the implantation period the proliferating layer of the implant and also the same layer of the condyle that had remained *in situ* displayed a good many labelled cells. This was interpreted by us as an indication that both in the implant and in the control condyle many cells in the proliferating layer were preparing for division. The question whether cells from the proliferating layer were in fact still differentiating into chondrocytes, was

answered by the experiments in which the period between the ^{3}H-TdR injection and the death of the animal was prolonged. 24 Hours after the injection administered on the 6th day of the implantation period, labelled chondrocytes were found on the articular side of the implants. These labelled cells were scattered over the various zones, but as yet they had not been eroded. In the implants from the rats that had been injected on the 5th day and fixed 48 hrs afterwards, labelled chondrocytes had arrived at the erosion zone on the articular side. When the post-injection time increased, there was a corresponding scatter of the labelled cells over the various layers in the right-hand control condyles. At 48 hrs after injection labelled hypertrophying chondrocytes in the right-hand control condyles were still at a considerable distance from the erosion zone, though.

These findings show that the processes occurring on the articular side of the implants were essentially the same as those which had taken place in the control condyles that had remained *in situ*. Accordingly, in our view the observed longitudinal growth of the implants was the result of processes comparable to those which had occurred in the control condyles. There were, however, differences between the implants and their controls as regards the course of these processes. In the implants the maturation and erosion of the chondrocytes happened much more rapidly. In the implants, the whole process from differentiation via hypertrophy to break-down runned its course between 24 and 48 hrs, whereas in the controls it needed much more time because here the labelled cells were still far removed from the erosion zone.

What could be established with certainty was that the proliferation and the differentiation into chondrocytes likewise continued in the implants over the period investigated.

In the implants the articular layer contained remarkably few labelled cells. This was also true for the condyles *in situ*. Dale *et al.* (1963) as well as Blackwood (1966) report the same observation in normal non-implanted condyles. Blackwood advances two explanations: either the cells could be renewed independently of the deeper layers, possibly also in a different tempo; or the articular layer might be harder to reach for the ^{3}H-TdR label, which is only briefly available, so that a smaller number of cells would get labelled. The fact that also in the implants only slight cell-labelling was found, speaks in favour of the former assumption. The fact that labelling in the articular layer did not increase with longer post-injection periods seems to suggest that labelled cells do not emigrate from the proliferating layer to the artic-

ular layer. This was also the conclusion arrived at by the authors just mentioned.

The fact that 2 hrs after the ^{3}H-TdR injection labelled cells were found in the old cartilage on the side of the cut face is not easily explained. Already at an earlier stage of the investigation (Chapter 5) we had noticed the occasional presence of several nuclei in a lacuna of the hypertrophic cartilage in this zone. Judging from the clearly labelled cells it is likely that we have indeed cell division here. The reason for this division might be that the cartilage was not broken up in time or was incapable of further differentiation. Summarizing, we may say that our ^{3}H-TdR experiments have supplied convincing proof that after the implantation chondrogenesis took place in the mandibular condyles. The way in which this happened corresponded to that of the non-implanted control condyles. Hence this process may be held responsible for the longitudinal growth of the implants.

Chapter 8

EFFECT OF THE IMPLANTATION TIME-SPAN ON THE AUTOLOGOUSLY IMPLANTED MANDIBULAR CONDYLE

A. INTRODUCTORY

For the purpose of getting a better insight into the processes occurring during the 7-day implantation period, a number of condyles was implanted over a shorter period, viz. one of 3 days.
The condylar implantation experiments described in the previous chapters had always been terminated after 7 days. They had shown that during the implantation period new cartilage was indeed still being formed, but only slightly so. Obviously, the balance between production and break-down of chondrocytes has been disturbed. Already in Chapter 5 we had postulated that the cartilage might in the end entirely disappear. A further test of this hypothesis seemed appropriate. To this end we devised a few experiments with a longer implantation period. At the same time they would enable us to collect information on other aspects.

B. EXPERIMENTAL DESIGN

In 16 rats of 4 days autologous intracerebral implantation of the mandibular condyle was performed according to the method as described in Chapter 2 (2.C.3 and 2.C.4). 4 Implants were retrieved 3 days after the operation, 7 implants after 11 days, and 4 after 26 days.

C. FINDINGS

In the implants that had been introduced for not longer than 3 days, no evident macroscopical or microscopical differences were observed on comparing them with the 4-day controls discussed in Capter 5. We did have the impression that the amount of hypertrophic cells had increased and that the number of young chondrocytes had been reduced, but endochondral ossification had made no headway yet. The implants that were

retrieved after 11 days showed great macroscopical as well as microscopical similarity to the 7-day ones. On the articular side, however, as a rule no more than a small cluster of hypertrophic chondrocytes was found. The proliferating layer had become a good deal narrower. Also, extensive penetration of connective-tissue strands and blood vessels had occurred.

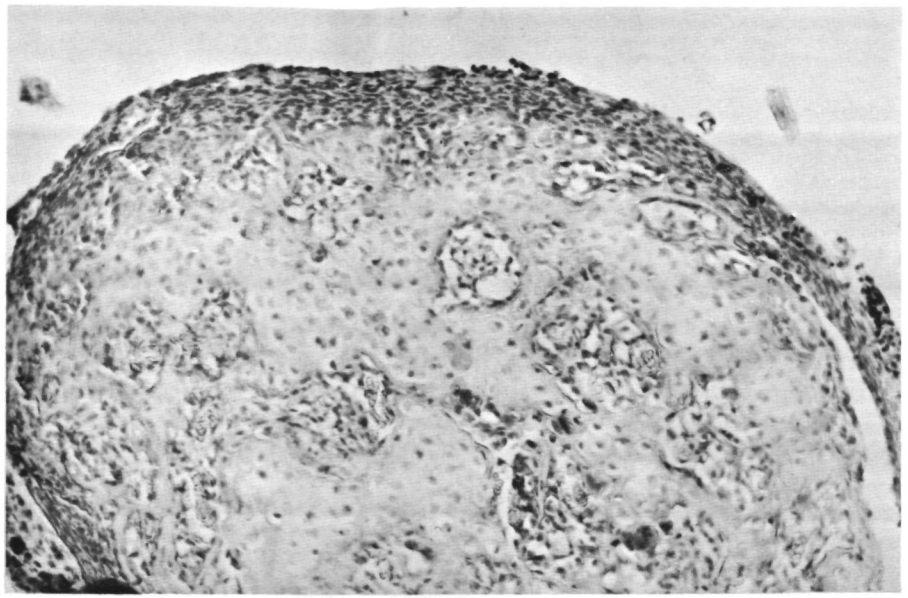

Figure 8.1
Section through an implant of the mandibular condyle, 26 days after autologous intracerebral implantation on the 4th day
At the end of this implant a remnant is visible of what might have been the proliferating layer. Here an area is observed of osteoid-like tissue pierced by large bloodvessels. The cartilage has entirely disappeared.
H.E.; Magnification: x 118.

The implants retrieved after 26 days appeared macroscopically not of greater length than the 7- and 11-day implants, but they were much thinner. Their shape did not any longer resemble that of the other implants. It was impossible even to distinguish between the articular and the cut face sides. Microscopically, all 4 implants were almost entirely ossified and for this reason had better be termed ossicles. At the ends some cartilaginous matrix remains were found, but otherwise they were made up of a compact bony layer, with a marrow cavity and bone mar-

row. The structure of this bony tissue corresponded to that of normal bony tissue of rats of that age. At the end of one implant a remnant was visible of what might have been the proliferating layer. Here, too, an area was observed of osteoid-like tissue, pierced by large blood-vessels (Fig. 8.1).

D. DISCUSSION AND CONCLUSIONS

The findings from the implants which had remained in the brain for longer than 7 days, showed the correctness of our supposition that over a longer implantation period the cartilage would entirely disappear. It is likely that around the 10th day after implantation chondrogenesis comes to a halt and that subsequently general ossification takes place. An investigation by means of ^{3}H-TdR might determine whether cell division in the proliferating layer is likewise brought to a standstill then. Increase of longitudinal growth is presumably limited to the first 10 days of implantation. We can only guess at the reasons why chondrogenesis should stop. It is relevant here to mention Holtrop's observation that costal cartilage implants containing the epiphyseal zones stopped growing after 2 weeks. The cartilaginous part did persist, however.

Chapter 9

EFFECT OF THE IMPLANTATION SITE ON THE AUTOLOGOUSLY IMPLANTED MANDIBULAR CONDYLE

A. INTRODUCTORY

In all the experiments so far described it was exclusively the brain that we used as site of implantation. Although a good many investigators hold the view that environmental influences on the fundamental processes in a skeletal tissue implant are only slight (Chapters 3.B and 5.A), it seemed appropriate to see whether this was also true for mandibular condyle implants. This was done by introducing a number of implants subcutaneously in the flank.

B. EXPERIMENTAL DESIGN

In 16 rats of 4 days unilateral condylectomy was performed and the mandibular condyle implanted into a subcutaneous pocket in the abdominal wall (Chapter 2.C.3 and 2.C.5).

C. FINDINGS

13 Animals survived the experiment in good condition. On retrieving the implants they appeared to have become attached to the fasciae and enveloped by a thick connective-tissue capsule. This prevented macroscopical observations and measurements. Owing to the thick capsule it was not possible, when preparing sections, to determine the sectional plane. This hampered microscopical assessment; we were unable to judge from the sections whether there had been any increase in length. We did ascertain, however, that in 5 out of the 13 implants endochondral ossification had occurred, while in 3 others in the middle of masses of hypertrophying cartilage the presence of small blood vessels and connective-tissue cells was observed and chondrocytes were seen being broken down. Endochondral ossification was even less orderly than in

the intracerebral implants described in Chapter 5. In the other implants the cartilage was almost entirely of the hypertrophic type, no ossification having set in as yet. Young chondrocytes were indeed present, but only in small numbers. The articular side could be distinguished from the cut face side by the presence of the various layers.

D. DISCUSSION AND CONCLUSIONS

As has been argued in Chapter 3, the experiment in which condyles underwent subcutaneous implantation was difficult to assess. The fact that in a number of implants endochondral ossification had set in and that it had made some headway in others, signifies that also in subcutaneous implants endochondral ossification may occur. If in a few implants we failed to observe this phenomenon it may be inferred that, compared with intracerebral implantation, subcutaneous implantation in the flank has a less positive vascularization, or that it takes a longer time for revascularization to set in.
From a comparison of the experiments it is clear that endochondral ossification is not limited to one particular site of implantation. The same holds good for chondrogenesis, if with less certainty, because it proved to be impossible to measure the subcutaneous implants. Hence there was no adequate way to establish for them any increase in length. This, together with the considerable inconsistency of the histological findings - probably to be traced back to a major variation in vascularization -, makes subcutaneous implantation little suitable for our purpose.

Chapter 10

GENERAL DISCUSSION

As has already been stated in Chapter 1, the present investigation attempts to find an answer to the following problems:

1. Is the isolated mandibular condyle (i.e. when taken outside its normal environment, as in the case of ***in vivo*** implantation) still capable of exhibiting perceptible growth?
2. If such growth should occur, is it comparable to that found in the normal situation?
3. If chondrogenesis should take place in the implanted mandibular condyle, does it differ - and if so, how - from chondrogenesis occurring in an implanted long bone?

Before dealing with these questions in detail, we shall briefly refer to prior experiments with mammalian mandibles (or parts of mandibles), mentioned in the literature. Their number is limited and they offer but little information bearing on the present issue.

Well-known is the experiment by Baker (1937), who performed homologous intramuscular and subcutaneous implantations of foetal rat mandibles in adult rats. He observed resorption of bony muscular attachments. Felts (1961) reported on the results of Ostergren (1958), his co-worker, with isologously subcutaneously implanted foetal mouse mandibles introduced into adult animals; he mentioned the unexpected failure of normal chondrogenesis in condylar cartilage.

Recently Koski ***et al.*** (1963, 1965) published the results of a sequence of experiments in which the mandibular condyle of 5-day rats was homologously implanted in litter-mates. Various types of implants and implantation sites were used; the brain was one of them. The implants remained 30, 60, 90 days in the hosts. Exact measurements of the removed implants were not carried out by these workers, although growth was confirmed.

It is difficult to make a comparison between the results of the experiments done by Koski ***et al.*** and those made by us. Their implantation period was considerably longer than ours. Only our supplementary

experiment, in which the implant remained in the brain for 26 days (Chapter 9), presents material for adequate comparison. Our implants were of a clearly similar character as those removed by Koski *et al.* after 30 days. Our experiments aimed especially at gaining insight into the processes relating to chondrogenesis. With the mandibular condyle implants in our experiments chondrogenesis came to a halt around the 10th day after implantation. This is the reason why Koski's experiments cannot give us a decisive answer for our purpose, all their implants having resided too long in the hosts. Neither, we think did their experiments come quite up to their aims 'to cast additional light in the problem of the role of the condylar cartilage as a growth center'.* These workers postulate that many of the bone cells found in their implants originate from tissues of the host. They assume that the stimulus for such bone formation derives from the implants. In this connection they use the term 'induction', as do many with them (Urist and McLean (1952), Chalmers and Ray (1959), Lacroix (1951), Ray and Sabet (1963), Arora and Laskin (1964). It is possible that Koski's were directed more towards the investigation of the possible potential for bone production by the mandibular condyle. We should bear in mind, however, that in long-term implantations all skeletal-tissue implants become ossified. Our preference for the brain as a site of implantation is shared by Koski *et al.* Finally, it may be underlined that their implantations were homologous, ours autologous.
There may be a link between some of our own observations and the investigation undertaken by Ronning (1966), who placed mandibular condyle implants of 5-day rats immediately underneath the cranial vault. On the basis of his findings he suggested that an osteogenic influence emanates from the dura mater. In fact, in 1 of our costal cartilage implants and in 2 condylar implants that were retrieved near the cranial vault, an abnormally large bone formation was established.

The isolated mandibular condyle, taken out of its normal environment, is still capable of manifest growth. Of this the experiments of Chapter 5 supply evident proof. The same is even more true of implanted long bones (Chapter 4). With them the shape of the implant continues to show greater similarity to the control specimen than is the case with the condylar implants; the latter become considerably thinner, whereas there is evident increase in length (Figs. 5.3 and 5.4). It is worth noting that

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* Koski, K., Makinen, L. (1963). Growth potential of transplanted components of the mandibular ramus of the rat, p.296. Finska Tandläk.Förh. 59:296-308.

when the sum total of the length of the implanted condyles and of the corresponding condylectomized mandibles is compared with the length of the control mandibles, only little difference is found. Apart from the condyle, the condylectomized mandible would correspond all but completely with its counterpart of the other side, the latter in its turn not differing demonstrably from the mandibles of the control animals.
One gets a strong impression that the growth of the rest of the mandible in the species and period studied by us is largely independent of that of the condylar process. The structure of the implants on the articular side showed great similarity to that of the condyles *in situ*. All the layers and zones were present, even if all were narrower. We saw the same phenomenon in the long bones that were examined, with the exception of the fact that the trabecular zone was much wider.

As regards the question whether the growth which occurred in the condylar implants is comparable to that taking place in the normal situation, here our experiments in which tetracycline marking and ^{3}H-TdR labelling were used (Chapters 6 and 7), have supplied the most information. From the implantation experiments discussed in Chapter 5 it had already become evident that the condylar implants had increased considerably in length and that endochondral ossification had set in. On the other hand, the balance between production and break-down of the chondrocytes had obviously been disturbed, a fact which, as said above, led to the termination of chondrogenesis around the 10th day after implantation. A curious phenomenon was that also on the side of the cut face in all implants cartilage was found, separated from the cartilage on the articular side by a wide zone of endochondral ossification (Fig. 5.8). It proved possible from the results of the implantation experiments combined with tetracycline marking, to throw more light on this phenomenon which we termed the 'cut face effect'. It appeared that the cartilage was the same that was present on the cut face at the moment of implantation. From this we inferred that the processes responsible for longitudinal growth in all probability took place on the articular side of the implant. From further experiments with ^{3}H-TdR labelling we were able to conclude that it is cells of the proliferating layer which divide and next differentiate into chondrocytes. It deserves notice that the chondrocytes in the implants underwent much more rapid maturation and erosion than those in the condyles *in situ*, which latter in this respect were used as controls. It is not unlikely that a more intense vascularization should be at the root of this. Why the chondrogenesis should come to a stop, is not clear.

As regards a comparison of the behaviour of the implanted mandibular condyle with that of the implanted long bone - our third question - the following may be said: In the implanted long bones the epiphyseal cartilage continues to exist, be it in more narrow zones. Even after longer implantation periods than ours, epiphyseal cartilage may be found (Felts, 1961).
Holtrop (1964), on the basis of her re-implantation experiments with costal cartilage implants, assumes that the bony part formed during implantation exerts at a certain moment an inhibitory influence on chondrogenesis. In how far this phenomenon should play a major part in the processes around the condylar implants, is not clear.

Chondrogenesis in the condyle takes a different course than in the epiphyseal cartilage. In the condyle the cells from the proliferating layer differentiate into chondrocytes; it is in this layer that mitotic activity is centred. This is compatible with Weinmann and Sicher's (1964) view that cartilaginous growth in the condylar process is of a depositional character and that no interstitial growth has yet been demonstrated. In the epiphyseal disk things are different. Here it is the zone of cell columns which shows interstitial growth. In the mandibular condyle, then, it is the chondrogenic cells that are in division; in the epiphyseal cartilage it is the chondrocytes which divide.

When we consider our findings in the light of the various views on the significance of the condyle in the growth of the mandible, the following may be said:
We may assert that in all our kinds of implants endochondral ossification had occurred. In addition, the costal cartilage fragments with the zone of small cells and the zone of cell columns, the implants of the third metacarpal bone, and the mandibular condyle implants showed an increase in length, caused by the formation of new cartilage. This means that both processes, chondrogenesis as well as endochondral ossification, can occur independently of their normal sites in the skeleton. Between the mandibular condyle implants and the implants containing epiphyseal cartilage, there is, however, as indicated before, an important difference, based on the way in which chondrogenesis takes places.
In the epiphyseal cartilage the proliferation of chondrocytes occurs in the columnar zone. The proliferating layer of the mandibular condyle is the production site for chondrogenic cells, which subsequently differentiate into chondrocytes, without presumably any further division

following.
In the mandibular condyle implants, probably both the activity of the proliferating layer and the differentiation into the chondrocytes had been more and more reduced or retarded, whereas the erosion of hypertrophic chondrocytes had, in all likelihood, been speeded up. The combination of these two factors may have been instrumental in causing the rapid disappearance of the cartilage on the articular side. Which of the factors mentioned has been preponderant, is still an open question. Perhaps a quantitative examination might present us with a solution here.

In the mandibular condyle implants the stimuli essential to the continuation of chondrogenesis were found to be increasingly scarce. About the nature of these stimuli and the way in which they work, nothing is known. Now there is no need to try and account for them direct in terms of functional mechanical factors. An indication that locally stimulating factors are operating in the process of chondrogenesis found in epiphyseal cartilage might be seen in Holtrop's (1966) recent finding that when two epiphyseal cartilage implants are introduced one behind the other, one of them will display considerably more growth than if it had been implanted alone. Here we also mention the observation made by Hoyte and Enlow (1966), that in sites of muscle attachments both resorption and deposition can occur independently. This they attribute to what they call the 'mechanism of local control'. They have no idea how it works, but think it is not solely a matter of muscle activity, that is, of mechanical functional influences.

These considerations made us suppose that the processes which caused an increase in length of the condylar implants, had occurred in the implants themselves. The degree of activity of these processes and their maintenance are, however, highly dependent on factors which obviously are only present in a normal environment.

On implantation the capacity of the epiphyseal cartilage to grow turns out to be less impaired. The differences in behaviour on implantation between epiphyseal cartilage and the mandibular condyle probably go back either to a relatively stronger intrinsic capacity for growth of the epiphyseal cartilage or to a relatively larger influence of the extrinsic factors on condylar growth, or to a combination of both.
With a normally functioning epiphyseal cartilage and mandibular condyle the extrinsic factors are likely to be more divergent than in our experiments. This is a major obstacle in transferring the above conclusion

to the natural situation. For this reason we would like to content ourselves by stating that in the process of growth of the mandibular condyle the intrinsic factors will probably be of relatively lesser importance than for the growth of the epiphyseal cartilage. With condylar growth it is the extrinsic factors that may be expected to carry the greater weight.

It appears then that in the rat, during the age period studied it is the chondrogenesis in the mandibular condyle which is largely responsible for the increase in length of the ramus. In other respects, however, mandibular development is but little affected by it. The extent of longitudinal growth and the change in shape of the mandibular condyle is largely dependent on extrinsic factors, but how the latter exert their effect is not clear. It is likely, however, that the influence of such factors is intensified with increasing age.

SUMMARY

Opinions on the significance to be attached to the growth of the mandibular condyle are widely divergent. According to Weinmann and Sicher (1955, 1964), and many others, its growth is directly responsible for the growth of the mandible and hence indirectly for the growth of the entire viscerocranium. In this view condylar growth takes up a key position. In contradistinction to this are found the views of Moss (1960, 1962, 1964), who argues that the processes of condylar growth are no more than a response to the growth of the surrounding parts, the functional matrix. According to him, condylar growth is primarily dependent on factors outside the skeletal tissues.

Because to the best of our knowledge no earlier research in this direction had been done, we decided to undertake a study of the growth of the mandibular condyle and its relevant processes by means of a series of *in vivo* implantation experiments. The following questions were our starting-point:

1. Is the isolated mandibular condyle (i.e. when taken outside its normal environment, as in the case of *in vivo* implantation) still capable of exhibiting perceptible growth?
2. If such growth should occur, is it comparable to that found in the normal situation?
3. If chondrogenesis should take place in the implanted mandibular condyle, does it differ - and if so, how - from chondrogenesis occurring in an implanted long bone?

In Chapter 2 a survey is given of the material and methods used. All operations were performed on 4-day-old rats; nearly always an implantation period of 7 days was adhered to.

We started on a pilot experiment with epiphyseal costal cartilage, in order to test the devised techniques as well as the suitableness of the brain as a site for implantation. By working with two types of implants, of different histological structure, we obtained additional information about the various cartilaginous zones (Fig. 3.2). Type I implants con-

tained small cell cartilage and the columnar zone; type II consisted mainly of a fragment of the columnar zone and the zone of hypertrophying cartilage. Out of the 20 type I implants introduced homologously (among litter-mates), 19 showed marked growth in length; the entire process of endochondral ossification had begun again. Type II implants, which were introduced both homologously (6 specimens) and autologously (15 specimens), did exhibit ossification, but no growth in length. From these pilot experiments it was clear that the intracerebral site of implantation - also because of the intense vascularization of the implants there - was most suitable for our purpose. Besides, the connective-tissue capsule formed around the implants was so thin as to make exact measurements of the implants possible. The mortality rate of this experiment made us decide to continue the investigation exclusively with autologous implantations. Nearly all type II implants and a few of type I exhibited an impaired development of the cells on the cut face, which we have termed 'cut face effect'.

This raised the question whether the cut face effect would also occur if the links between the various zones were not severed. The matter was further investigated by intracerebral implantation in 22 rats of the third metacarpal bone *in toto*. Measurements indicated that the implanted metacarpals had grown considerably. In comparison with the controls the implants remained smaller and they deviated in shape. This was ascribed to a reduced interstitial growth of the cartilage. The histological picture of the epiphyseal cartilage in the implants appeared to be practically identical to that found in the controls. The articular cartilage had indeed developed, but remained much thinner.

Next, in 24 rats autologous intracerebral implantation of the mandibular condyle was performed, in combination with unilateral condylectomy. By the side of a considerable increase in length these implants showed a decrease in width. The formation of a condyle comparable in shape to a normal one, did not occur. If we disregard its condylar part, the shape of the condylectomized left-sided mandible practically corresponded with its counterpart, the latter not detectably differing from the control mandibles. The sum of the length of the implant and the length of the condylectomized left-sided mandible measured in the direction of condylar growth constitued 91.6% - 94.9% of the length of the non-operated right mandible. A histological examination showed that in the implants endochondral ossification had developed. All the zones seen in a normal mandibular condyle were present, if considerably narrower. Here, too, there was impaired development of the hypertrophic chondrocytes on the side of the cut face, corresponding to the cut face effect we had found

in costal cartilage implants (Fig. 5.8).

In order to obtain additional data about the mode of growth of the mandibular condyle implants, we applied the bone marking technique (Chapter 6).
In 4 animals, prior to mandibular condyle implantation, the calcified cartilage on the side of the cut face was marked with tetracycline. After an implantation period of 7 days, the tetracycline turned out to be still present at the cut face of the implants. Hence we inferred that this cartilage corresponded to the cartilage which had been present at the cut face at the moment of implantation. We also concluded that the striking growth in length must have occurred on the articular side of the implants.
These experiments, however, did not supply us with the answer to the question as to what cells are to be held responsible for longitudinal growth. For further study we supplemented our implantation experiments by the technique of autoradiography.
By injecting the animals with tritiated thymidine during the period that the implants remained in the brain, we found that the way in which chondrogenesis occurred in the implants was comparable to how it happened in the condyles that had remained *in situ* (Chapter 7). Hypertrophic chondrocytes eroded sooner, however, a fact attributed to the intense vascularization of the implants.
It seemed worth while to examine the processes of growth by means of long-term implants. When in 7 animals the period of implantation was lengthened to 11 days, it was established that chondrogenesis came to a stop on approximately the 10th day. Experiments with 4 rats and an implantation period of 26 days learned that all cartilage disappeared and the implants ossified.
The experimental part of the present thesis ends with an experiment designed to find out in how far similar processes would happen at other sites of implantation. For this purpose 13 implants were subcutaneously introduced in the flank. We learned that under these conditions endochondral ossification and probably chondrogenesis may indeed develop in the implants, but that this is a less suitable site for implantation of the mandibular condyle.
From our implantation experiments with the mandibular condyle it was found that after intracerebral autologous implantation the isolated condyles still showed evident growth, and that this mode of growth is similar to that of a condyle *in situ*. But as the balance between production and break-down of the chondrocytes evidently had been disturbed,

chondrogenesis came to a halt around the 10th day after implantation, as mentioned above.
Between the mandibular condyle implants and the implants containing epiphyseal cartilage there is an important difference, which goes back to the way in which chondrogenesis takes place. In the epiphyseal cartilage the proliferation of chondrocytes occurs in the zone of cell columns. The proliferating layer of the mandibular condyle is the production site for chondrogenic cells, which subsequently differentiate into chondrocytes without presumably any further division following.
These findings and considerations made us suppose that the processes responsible for the increase in length in the condylar implants are to be found in the implants themselves. The degree of activity of these processes and their maintenance are, however, highly dependent on factors which obviously are only present in a normal environment. On implantation the capacity of the epiphyseal cartilage to grow is considerably less impaired than that of the cartilage of the condylar implants. The differences in behaviour on implantation between epiphyseal cartilage and the mandibular condyle probably go back either to a relatively greater intrinsic capacity for growth of the epiphyseal cartilage or to a relatively larger influence of the extrinsic factors on condylar growth, or to a combination of both.

It appears then that in the rat, during the age period studied it is the chondrogenesis in the mandibular condyle which is largely responsible for the increase in length of the ramus. In other respects, however, mandibular development is but little affected by it. The extent of longitudinal growth and the change in shape of the mandibular condyle is largely dependent on extrinsic factors, but how the latter exert their effect is not clear. It is likely, however, that the influence of such factors is intensified with increasing age.

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STELLINGEN

1

De chondrogenese in de processus articularis mandibulae wordt beheerst door in dit deel van het skelet zelf aanwezige factoren; de mate en de richting waarin de chondrogenese plaats vindt worden echter sterk bepaald door factoren daar buiten.

2

In een rat zijn gemerkte chondrocyten in het epiphysaire kraakbeen aantoonbaar, indien 2 uren voorafgaande aan de histologische fixatie van dat kraakbeen getritieerd thymidine is geinjiceerd.
In de processus articularis mandibulae zijn onder deze omstandigheden in het kraakbeen géén gemerkte chondrocyten waar te nemen, maar zijn er gemerkte chondrogene cellen in de proliferatielaag te vinden.
Deze waarnemingen vormen een steun voor de opvatting van Weinmann en Sicher, dat het kraakbeen in de processus articularis mandibulae niet interstitieel groeit maar door appositie.

Weinmann, J.P., Sicher, H. (1964) in: The temporomandibular joint (ed.B.G.Sarnat) Springfield, Illinois Charles Thomas.

3

De opvatting als zou de aanleg van de mandibula uitgaan van één linker en één rechter ossificatiecentrum is onvoldoende gefundeerd.

4

Dat Enlow en Harris voor de groeirichting van de processus articularis mandibulae een ander gemiddelde vonden dan Björk is hoofdzakelijk terug te voeren op het verschil in samenstelling van het onderzochte materiaal.

Björk, A. (1963) J.Dent.Res. 42:400-411.
Enlow, D.H., Harris, D.P. (1964) Amer.J.Orthodont. 50:25-50.

5

Moss heeft de morphologische begrenzing van de door hem geïntroduceerde 'functional matrix' onvoldoende aangegeven.

Moss, M.L. (1962) in: Vistas in Orthodontics
(ed.B.S. Kraus, R.A. Riedel) p.85-98 Philadelphia, Lea & Febiger.

6

In het algemeen dient het transponeren van conclusies uit dierproeven naar situaties bij de mens voorzichtig te geschieden. In het biezonder geldt dit voor experimenten met het tandkaakstelsel van rodentiae.

7

Bij een betere kennis van de gebitsontwikkeling zouden overbodige frenulumextirpaties minder worden uitgevoerd.

8

Een praktisch naslagwerk, waarin door uitbreidingen en vervangingen de recente ontwikkelingen in de tandheelkunde op de voet worden gevolgd, kan een belangrijke bijdrage leveren tot het postuniversitaire onderwijs.

9

Bij de opleiding tot arts en tandarts dient meer aandacht geschonken te worden aan de bestudering van het normale.

10

Bij het opstellen van plannen voor de ruimtelijke ordening binnen Nederland dient aan het totaal aantal inwoners een limiet gesteld te worden.

Stellingen behorende bij het proefschrift van
H.S. Duterloo

Nijmegen, mei 1967